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(For references see pages 460-461)

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Vitamin A^{1,2}

Age at which symptoms appear	Genetics	Reference
From birth Childhood Up to 30 years or later	(a) Recessive (b) Recessive (c) Uncertain	3
1-2 years Late childhood or adulthood	(a) Recessive (b) Uncertain	4
Infantly early childhood	Complex	5
Infancy childhood adulthood	Usually dominant	5, 6

et al. (Eds.), *The Metabolic Basis of Inherited Disease*, New York, 1966, page 539.
 ANDRUEY et al. (Eds.), *The Metabolic Basis of Inherited Disease*, New York, 1966, page 429.
 (1966), 45, 77 (1966).

is excrete only small amounts of 17- α -OH and they show hardly any increase in corticosterone causes sodium retention, use of the arterial hypertension seen in

steroid dehydrogenase is rare; this gesterone from pregnenolone. As with on, the result is loss of salt in the urine, differs from inborn adrenal hyperplasia affects differentiation of the external

steroid 17 α -hydroxylase has been reported produced excessive amounts of corticosterone, with consequent arterial

inborn adrenal hyperplasia are probably autosomal recessive gene.

and Gland, Lea & Febiger, Philadelphia, 1961;
 ANDRUEY et al. (Eds.), *The Metabolic Basis of Inherited Disease*, New York, 1966, page 635.
 5, 1946 (1966).

Chemistry³

Vitamin A and the carotenes are soluble in fats, insoluble in water, and readily oxidized. In the absence of oxygen they are stable to acids, alkalis and heat. On the *cis-trans* isomerism of the carotenes and vitamin A see ZECHMEISTER⁴. For structure and properties of vitamin A and related compounds see the table on pages 458-459.

Assay

Biological⁵. Mainly by the standardized growth test on vitamin A-deficient rats.

Chemical⁶. Spectrophotometrically in pure solution (vitamin A at 328 nm, carotenes at ca. 450 nm) or colorimetrically, for instance with antimony trichloride (CARR-PRICE reaction); in biological material chromatographically after suitable extraction.

Units

Vitamin A. 1 International Unit (IU) = 0.344 μ g *all-trans* vitamin A₁ acetate = 0.300 μ g *all-trans* vitamin A₁; 1 US Pharmacopeia (USP) Unit = 1 International Unit.

Carotenes. 1 International Unit (IU) = 0.6 μ g β -carotene, equivalent in activity to 1 IU vitamin A.

Biogenesis^{7, 8}

The carotenes are synthesized by the higher plants, algae and photosynthetic bacteria and are found in concentrated form in the chloroplasts. Acetate is converted by condensation and decarboxylation into isopentenyl pyrophosphate, from which a C₄₀ terpenol pyrophosphate arises by condensation. This substance gives rise by further condensation to a carotenoid precursor with 40 C-atoms, probably phytoene. The various carotenes arise by dehydrogenation, cyclization, isomerization, hydration and hydroxylation. Carotenes with a β -ionone ring are broken down in the animal organism to vitamin A, more probably by fission in the middle of the chain than by successive β -oxidation from the end of the isoprenoid chain⁹. In the liver oils, vitamin A is present in the esterified form.

Intake and excretion

In the USA the daily diet contains ca. 7500-10000 IU (2.3-3 mg) of vitamin A^{10, 11}. About a half of the apparent vitamin intake is in the form of the provitamin.

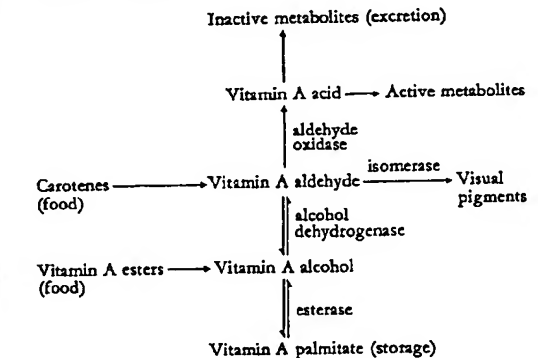
The vitamin A esters are hydrolysed in the lumen of the duodenum, probably by esterases of the pancreatic juice. Bile is necessary for absorption of the carotenes but not of vitamin A; the absorption of the carotenes is promoted by fats. In the wall of the duodenum and upper jejunum the carotenes are broken down to vitamin A, with vitamin A aldehyde as an intermediate product^{9, 12}. In the intestinal wall vitamin A is probably mainly esterified with palmitic acid, the ester being transported by the lymph^{13, 14}. The carotenes are less easily absorbed than vitamin A and part of those ingested appears in the faeces. Under favourable conditions the biological activity of β -carotene amounts to about half that of vitamin A.

The vitamin A level in blood is at its highest about 4 hours after giving the vitamin². The mean normal levels in serum are 850 μ g carotene/l and 324 μ g vitamin A/l (see page 609). In the serum, vitamin A is normally present to the extent of about 90% as the alcohol, the remainder as the ester; shortly after intake of vitamin A, however, the proportion of ester is increased¹⁵. Freshly absorbed vitamin A esters are transported by the lipoproteins S_{β} 10-400. Vitamin A alcohol is probably bound to albumin in the serum¹⁴. In breast milk vitamin A is about 90% esterified.

Vitamin A is stored in the liver, the carotenes mainly in the fatty tissues. About 90% of the whole vitamin A of the body is stored in the liver; the liver reserves (up to 300 μ g vitamin A per gramme liver or more¹⁶) are sufficient to meet the body's requirements of the vitamin for one year or more². These reserves, however, are rapidly used up in infections, hyperthermia and poisoning². 90-95% of the vitamin A in the liver is present as palmitate, the remainder as aldehyde and alcohol^{14, 18}. When any organ requires vitamin A, the esters in the liver are hydrolysed and the free alcohol transported by the blood to where it is required. In the tissues, particularly in the liver, vitamin A alcohol and aldehyde are rapidly oxidized to vitamin A acid; this substance is not stored, however, but rapidly broken down^{6, 17}. An active metabolite of vitamin A acid has recently been identified but its composition is unknown¹⁶. After adminis-

tration of tagged vitamin A to rats, radioactivity can be detected in the bile, urine and faeces²; the presence of vitamin A and/or its metabolites in bile points to enterohepatic circulation of the vitamin¹⁸.

Metabolism of vitamin A



Function

Vitamin A is of great importance for maintenance of health and life, normal growth, the visual process and reproductivity. It appears to be necessary for the stability of the lipoprotein membrane of the cell and of the subcellular particles¹⁹.

Vitamin A alcohol can be converted in the body to the aldehyde and acid and has a specific effect on the ability to reproduce. Vitamin A aldehyde is the prosthetic group of the visual pigments. Vitamin A acid, while very active in maintaining growth, is not capable of maintaining reproductivity.

Metabolic functions of vitamin A²⁰

Active form	Biochemical reaction	Clinical effect
Vitamin A alcohol or other active form	Unknown	Reproduction in both sexes
Vitamin A aldehyde	Reaction with opsin	Visual process
Vitamin A acid or other active form	Liberation of proteolytic enzymes	Breakdown of cartilage
	Synthesis of mucopolysaccharides	Stimulation of mucous secretion in the epithelium
	Synthesis of corticosterone	Lesions in the adrenal cortex, interference with gluconeogenesis in deficiency states

The vitamin A aldehydes retinal and dehydroretinal form together with the protein opsin the light-sensitive pigments in the rods (night vision) and cones (day and colour vision) of the retina. The four main types of pigment^{21, 22} are rhodopsin (retinal + rod opsin) in terrestrial and aquatic animals, iodopsin (retinal + cone opsin) in terrestrial animals, porphyropsin (dehydroretinal + rod opsin) in fresh-water animals, and cyanopsin (dehydroretinal + cone opsin). Absorption of light by the visual pigments causes transformation of the 11-*cis* isomers of retinal or dehydroretinal into the *all-trans* form. The potential difference induced by this isomerization is responsible for the registration in the brain of the visual impressions from the rods and cones. 11-*cis*-Retinal is not only the prosthetic group of the visual pigment in the rods but also of the red- and green-sensitive pigments in the cones of the human eye²³. The *cis-trans* isomerization of retinal proceeds as follows²¹:

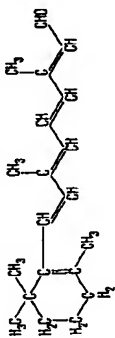
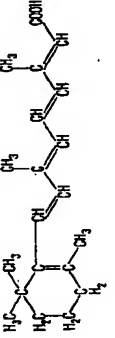
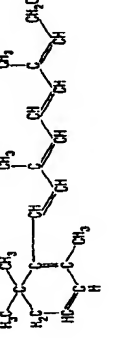
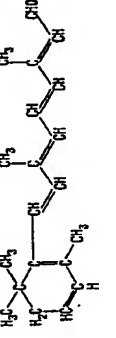
(continued on page 460)

Structure and properties of vitamin A and related compounds

Names*	Formula and mol. wt.	Structure	Physical properties	Occurrence	Relative activity
α -Carotene	$C_{40}H_{56}$ 536.89		Violet to red crystals M.p. 187 °C (benzene/methanol)	Palm oil, mountain ash berries	50
β -Carotene	$C_{40}H_{56}$ 536.89		Violet to red crystals M.p. 180 °C	Plants, fruits	100
Cryptoxanthene (3-hydroxy- β -carotene)	$C_{40}H_{54}O$ 552.89		Red platelets M.p. 158 °C	Maize	50
Vitamin A ₁ (<i>all-trans</i>) (retinol*, azetophthol)	$C_{20}H_{30}O$ 286.46		Yellow prisms M.p. 62-64 °C	Liver of marine fish	100
9- <i>cis</i> -Vitamin A ₁ (<i>iso-a</i>)	$C_{20}H_{30}O$ 286.46	As <i>all-trans</i> vitamin A ₁ but with double bond at C-9 in <i>cis</i> configuration	Yellow prisms M.p. 82 °C	-	21
11- <i>cis</i> -Vitamin A ₁ (<i>neo-β</i>) (11- <i>cis</i> -retinol)	$C_{20}H_{30}O$ 286.46	As <i>all-trans</i> vitamin A ₁ but with double bond at C-11 in <i>cis</i> configuration	Orange-yellow oil	Retina	23

13- <i>cis</i> -Vitamin A ₁ (<i>neo-a</i>)	$C_{20}H_{30}O$ 286.46	As <i>all-trans</i> vitamin A ₁ but with double bond at C-13 in <i>cis</i> configuration	Yellow prisms M.p. 58 °C	Fish liver	75
Vitamin-A ₁ aldehyde (<i>all-trans</i>) (retinal*, retin- aldehyde*, β -retinene, retinene)	$C_{20}H_{28}O$ 284.45		Orange prisms M.p. 58 °C	Citrus fruits, green vegetables, liver	91

NAME	STRUCTURE	MOLECULAR WEIGHT	BOILING POINT	MELTING POINT	ANALYSIS
(11- <i>cis</i>)		286.46			
11- <i>cis</i> -Vitamin A ₁ (<i>me-b</i>) (11- <i>cis</i> -retinol)	As <i>all-trans</i> vitamin A ₁ but with double bond at C-11 in <i>cis</i> configuration	C ₅₀ H ₁₀₀ O 286.46		Orange-yellow oil	Retina

13- <i>cis</i> -Vitamin A ₁ (<i>me-a</i>)	As <i>all-trans</i> vitamin A ₁ but with double bond at C-13 in <i>cis</i> configuration	C ₅₀ H ₁₀₀ O 286.46		Yellow prisms M.p. 58 °C	Fish liver
Vitamin A ₁ aldehyde (<i>all-trans</i>) (retinal*, retin- aldehyde*, β-retinene, retinene)		C ₅₀ H ₁₀₀ O 284.45		Orange prisms M.p. 58 °C	Citrus fruits, green vegetables, liver
11- <i>cis</i> -Vitamin A ₁ aldehyde (<i>me-b</i>) (11- <i>cis</i> -retinal)	As <i>all-trans</i> vitamin A ₁ aldehyde but with double bond at C-11 in <i>cis</i> configuration	C ₅₀ H ₁₀₀ O 284.45		Orange prisms M.p. 64 °C	Eyes of crustacea
Vitamin A ₁ carboxylic acid (<i>all-trans</i>) (retinic acid*)		C ₅₀ H ₁₀₀ O ₂ 300.44		Yellow needles M.p. 179 °C	Tissues?
Vitamin A ₂ (3-dehydroretinol*)		C ₅₀ H ₁₀₀ O 284.45		Yellow needles M.p. 63-65 °C	Liver of freshwater fish
Vitamin A ₂ aldehyde (3-dehydroretinal*, 3-dehydroretinalde- hyde*, α-retinene, retinene)		C ₅₀ H ₁₀₀ O 282.43		Orange-red prisms M.p. 78 °C	Retina in fish

* Trivial names recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry [Biochim. biophys. Acta (*Amst.*), 107, 1 (1965)].

(For references see page 464)

e typical lesions of vitamin A deficiency²⁸ is or keratinization of various membranes (ia) and the formation of defective bony growth. The most sensitive test for vitamin A deficiency is the dark adaptation of the eye²⁹; A concentration in the serum is less reliable than until the body's reserves are exhausted in countries of South America, Asia and ill one of the commonest causes of blindness manifestations of vitamin A deficiency conjunctiva and roughness of the skin due to follicles. In animals vitamin A deficiency is a cause of infertility and

should be treated by giving vitamin A in (corresponding to ca. 30 ml liver oil). Initially higher doses (5000 IU/kg body³⁰). When liver oils are given in large ingested in toxic amounts, even though it n in A in large doses diminishes the toxic

ith high doses of vitamin A (for instance ay in children) may result in toxic sym- pectia, affections of the skin and mucosa, diaphyses of the limbs, anaemia, enlarge- in and headache. All these symptoms are apidly on cessation of the treatment³¹. In amin A may interfere with bone develop- re fusion of the epiphyses³². nin A is given prophylactically to new- n daily doses of 7500–10000 IU. Because toxication, however, it is better to restrict) IU per day³³. cation has been observed following the at liver, which contains 20000 IU per contains 4400 IU vitamin A per gramme,) IU per gramme³⁴.

), *The Vitamins*, vol. 1, Academic Press, New e, T., *Vitamin A*, Elsevier, Amsterdam, 1957; and Metabolism, *Vitamin and Horm.*, 18, 289 and, in BEATON and MCHENRY (Eds.), *Nutrition*, e York, 1964, page 1. WOHL and GOODHART (Eds.), *Modern Nutrition* 1, Lea & Febiger, Philadelphia, 1964, page 341. *vitaminoids*, Elsevier, Amsterdam, 1950; FRYE- (Ed.), *Biochemisches Taschenbuch*, 2nd ed., part 1, : 358. *Isomerit Carotenoids, Vitamin A and Arylpolyenes*, *Ann.*, 18, 341 (1960). *Ann.*, 18, 295 (1960); GEFNER, F., *Chemisch-physi- othoden*, 5th ed., Enke, Stuttgart, 1965, page 5. *Isomerit of Vitamins and Related Compounds*, *Acta-* 63, page 270. : 281 (1964). : 149, 879 (1965); GOODMAN et al., *J. Biol. Chem.*, 257 (1963). *Recommended Dietary Allowances*, 7th ed., Nation- National Research Council, Publication 1694,

Ann., 18, 371 (1960). *J.*, 88, 534 (1963). *Nutr. Diet.*, 5, 209 (1965). *Ann.*, 18, 387 (1960). : 411 (1964). *ophy. Acta (Amst.)*, 107, 120 (1965). *Ann.*, 201, 1222 (1964). : 104, 156 (1964). : 11 (1962); Colloquium on aspects of vitamin A : (1964). : 18, 417 (1960). *Ann. Rev. Physiol.*, 25, 433 (1963). : 200, 37 (1963). : 191, 1310 (1961); DUNCAN and BRIGGS, *Arch.* Group, *Wld Hlth Org. techs. Rep. Ser.*, No. 362 *Ser. med. Res. Coun. (Lond.)*, No. 264 (1949). : 23, No. 3, 29 (1964), quoted in *Nutr. Rev.*, 22,

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29 McLAREN, D. S., *Nutr. Rev.*, 22, 289 (1964).
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33 PEASE, C. N., *J. Amer. med. Ass.*, 182, 980 (1962).
34 TUNELL et al., *Acta paediat. scand.*, 54, 61 (1965).
35 RODAHL and MOORE, *Biochem. J.*, 37, 166 (1943).
36 NOTES, *Nutr. Rev.*, 19, 318 (1961).

Vitamin D^{1,2}

Chemistry³

The D vitamins are odourless crystals, soluble in fats and fat solvents, insoluble in water, and only slightly photosensitive. All show a wide absorption band at 260–290 nm. For structure and properties of vitamin D and related compounds see the table on pages 462–463.

Assay

Biological⁴. By its preventive or curative effect on young rats with experimental rickets.

Chemical⁵. Spectrophotometrically in pure solution at 265 nm; the individual D vitamins cannot be distinguished. Colorimetrically with antimony trichloride, in the presence of vitamin A after chromatographic separation. The individual D vitamins can be separated by column, paper or thin-layer chromatography.

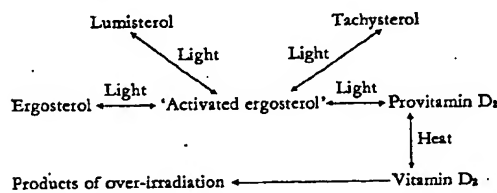
Units

1 International Unit (IU) = 0.025 µg crystalline vitamin D₃. 1 US Pharmacopeia Unit = 1 International Unit (see also International Standard for Vitamin D₃, page 763). The earlier international unit equivalent to 1 mg of standardized irradiated ergosterol dissolved in a vegetable oil is now obsolete.

Biogenesis

Vitamin D₃ occurs solely in the higher animals and is formed photochemically from 7-dehydrocholesterol (provitamin D₃, animal provitamin). 7-Dehydrocholesterol is formed from cholesterol by a reaction that also takes place in the intestinal tissues⁶. Other D vitamins of known constitution and formed by ultraviolet irradiation at wave lengths of 275–300 nm are the following: D₂ from ergosterol, D₄ from 22-dihydroergosterol, D₅ from 7-dehydrostirosterol, D₆ from 2-dehydrostigmastanol and D₇ from 7-dehydrocampesterol.

The conversion of provitamin D into vitamin D by irradiation occurs as follows⁶:



In fish liver oils vitamin D is partly esterified. The origin of vitamin D in fish is not known. Plants contain substances with a strong antirachitic effect (ketone 250).

Intake and excretion

In man, vitamin D is ingested in foods in addition to being formed from 7-dehydrocholesterol in the skin under the effect of sunlight. The vitamin formed in the skin is very rapidly absorbed⁷ and transported almost quantitatively to the sites where it is required⁸. Bile appears to be necessary for the absorption of vitamin D ingested in foods. In rats, the vitamin is partly esterified during absorption and is transported by the lymph⁹. A similar process has also been observed in man¹⁰. In children the blood level is 860–2100 IU/l¹¹, in adults 700–3100 IU/l¹². With a daily oral intake of 50000–500000 IU, the blood level in adults rises to 90000–130000 IU/l¹². In the blood, the vitamin is transported bound to the α₂-globulins and albumin¹³. It is taken up by and metabolized in the liver, and is also present in other tissues such as the kidneys, intestine, adrenals and

bones^{13,14}. 24 hours after giving ¹⁴C-tagged vitamin D, 10% of the activity was found in the tissues and 20% in the faeces, the remaining 70% being present as breakdown products¹⁵. In rats, some vitamin D is excreted in the bile bound to taurine and glycine and then partly reabsorbed. In man, the urine contains no vitamin D as such but up to 2–4% of the radioactivity of ingested tagged vitamin D has been found there¹⁶.

The vitamin D content of the skin exposed to sunlight amounts to ca. 1 IU/cm². In the skin of the human back activation may reach the level of 15 IU/cm². 50–75% of the activity is found in the deeper layers of the epidermis and 25–50% in the parts of the corium adjacent to the epidermis¹⁷.

It has been suggested that the metabolic effect of vitamin D is due not to the vitamin itself but to a metabolite¹⁸. Such an active metabolite has been found in the nuclear fraction of the intestinal mucosa¹⁹. Recently a biologically active metabolite of vitamin D has been identified as 25-hydroxycholecalciferol²⁰.

Function

The activity of vitamin D is closely related to that of parathyroid hormone and of calcitonin, all three factors being necessary for maintenance of calcium balance and a normal serum calcium level^{21,22}. Vitamin D may be responsible for formation of the calcium transport system in bone cells; the control of serum calcium concentration by parathyroid hormone depends on the presence of vitamin D, whereas that by calcitonin does not²⁴. It has been shown that the physiological expression of the action of vitamin D on intestinal calcium transport and bone mineral mobilization probably involves DNA transcription into messenger RNA and protein synthesis²⁵. At the subcellular level, vitamin D causes release of the bound calcium in the mitochondria, probably as a result of its action on oxidative phosphorylation²⁶.

In rats, vitamin D has been shown to increase calcium absorption mainly in the intestine²⁷. A vitamin D-dependent calcium-binding protein has been isolated from the intestinal mucosa of chicks²⁸. There is evidence that vitamin D also favours the intestinal absorption of magnesium²⁹.

Vitamin D is also necessary for the development of normal bone and for the calcification of rachitic bone. The manner in which it acts is obscure but probably the defective calcification of newly formed bone in rickets and osteomalacia is due to a reduction of the calcium phosphate product in the tissue fluid surrounding the osteoid³⁰. The increase in the serum citrate level caused by vitamin D is probably due to its effect on calcium metabolism³¹.

It is not certain whether vitamin D has any effect on the renal transport of calcium; in any case this effect is small in comparison with that of parathyroid hormone³². Vitamin D is thought to favour the tubular reabsorption of phosphate³³.

Requirements and deficiency symptoms

In adults the body's needs of vitamin D are usually met by its own synthesis, provided this is not impaired by lack of daylight, as in night workers, miners, and people living in subpolar regions. During the period of active skeletal growth, in pregnancy and in lactation, there is an increase in both the calcium and vitamin D requirements, so that intake of exogenous vitamin D is advisable. In such cases, oral administration of 400 IU vitamin D per day is adequate³⁴ (see also pages 493–494).

The liver oils are rich in vitamin D: tunny fish 7000–50000 IU per gramme, cod 60–300 IU per gramme; the mammalian liver contains only small quantities of vitamin D, egg yolk 1.5–5 IU per gramme (values from DAM and SØNDERGAARD³⁵).

Deficiency of vitamin D causes rickets, of which osteomalacia is the adult form^{36,37}. Both diseases are marked by failure of mineralization in newly formed bone, leading to progressive demineralization and weakening of the skeleton. In children, bone growth is particularly rapid in the epiphyses and it is here that vitamin D deficiency is first manifested. In adults, on the other hand, there is slow breakdown of bone throughout the skeleton, so that the disease develops in a slower and more widespread fashion. Rickets and osteomalacia can be classified as follows³⁸:

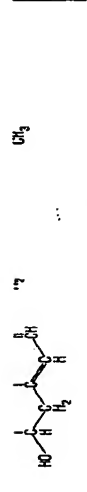
1. Rickets due to vitamin D deficiency
 - (a) vitamin D deficiency in food and lack of sunlight
 - (b) vitamin D deficiency in food with pigmentation of the skin
 - (c) impaired absorption (idiopathic steatorrhoea, coeliac disease)
2. Vitamin D-resistant rickets
 - (a) renotubular acidosis

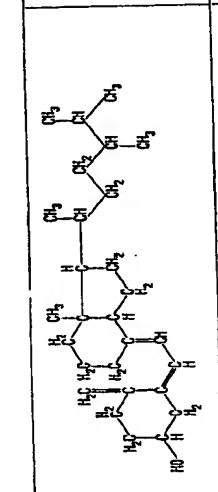
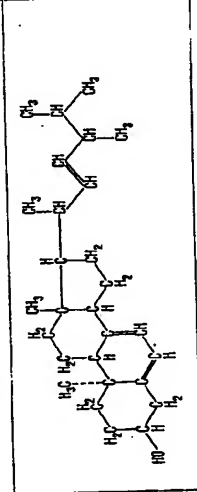
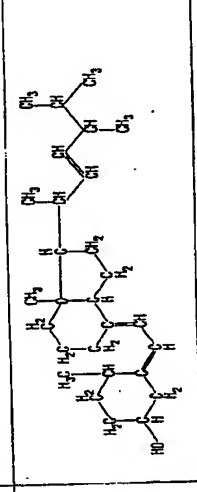

(continued on page 464)

Structure and properties of vitamin D and related compounds:

Names*	Formula and mol. wt.	Structure	Physical properties	Occurrence	Antirachitic activity
Ergosterol	$C_{28}H_{44}O$ 396.66		M.p. 168 °C (with 1½ H ₂ O)	Yeast, ergot, hens' eggs	None (provitamin D ₂)
7-Dehydrocholesterol	$C_{27}H_{44}O$ 384.65		M.p. 150 °C (anhydrous)	Higher animals, man	None (provitamin D ₃)
22-Dihydroergosterol	$C_{28}H_{46}O$ 398.68		M.p. 152 °C	Synthetic	None (provitamin D ₄)
Vitamin D ₂ (ergocalciferol*)	$C_{28}H_{44}O$ 396.66		M.p. 115-118 °C	Formed by irradiation of ergosterol	In rats similar to, in chicks and apes less than that of vitamin D ₃
Vitamin D ₃ (cholecalciferol*)	$C_{27}H_{44}O$ 384.65		M.p. 84-85 °C	Formed by irradiation of 7-dehydrocholesterol. In fish-liver oils, egg yolk, milk	Antirachitic

Vitamin D ₄	$C_{28}H_{46}O$ 398.68		M.p. 96-98 °C	Formed by irradiation of 22-dihydroergosterol	Rather less than that of vitamin D ₃
Ketone 250	$C_{27}H_{44}O_2$ 418.67		M.p. 73 °C	Plants, fish-liver oils	One-tenth that of vitamin D ₃

Vitamin D ₃ (cholecalciferol*)	C ₂₇ H ₄₄ O 384.65		M.p. 84-85 °C	Formed by irradiation of 7-dehydrocholesterol. In fish-liver oils, egg yolk, milk	Antirachitic
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Vitamin D ₄	C ₂₈ H ₄₆ O 398.68		M.p. 96-98 °C	Formed by irradiation of 22-dihydroergosterol	Rather less than that of vitamin D ₃
Kerone 250	C ₂₇ H ₄₄ O ₂ 418.67		M.p. 73 °C	Plants, fish-liver oils	One-tenth that of vitamin D ₃
Lumisterol	C ₂₈ H ₄₆ O 398.66		M.p. 118 °C	Formed by irradiation of ergosterol	None
Tachysterol	C ₂₈ H ₄₆ O 398.66		Oil, readily oxidizing in air	Formed by irradiation of ergosterol	None (hypercalcaemic effect)
Dihydrotachysterol	C ₂₈ H ₄₈ O 398.68		M.p. 125-127 °C	Synthetic	400 times less active than vitamin D ₃ (same hypercalcaemic effect)

* Trivial names recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry [Biochim. biophys. Acta (Amst.), 107, 1 (1965)].

- (b) FANCONI's syndrome
(c) primary vitamin D resistance
(d) renal insufficiency

The clinical symptoms of rickets are pain in the limbs, particularly the legs, genu valgum, bending of the long bones, thickening of the synchondroses of the ribs and of the joint epiphyses, and protuberance of the forehead. Tetany is also an occasional symptom. The radiological changes consist in widening of the epiphyses combined with disorganization of the epiphyseal disk, and appearance of a cup-shaped structure in place of the normal, distinct, straight boundary between metaphysis and epiphysis. The biochemical changes observed are slight lowering of the plasma calcium level, marked lowering of the plasma phosphate level, reduced urinary calcium excretion, increased phosphate clearance, increased phosphate excretion index and a rise in the plasma concentration of alkaline phosphatase; the calcium phosphate product in the plasma is lower than normal. One of the first signs of vitamin D deficiency is an increase in the amino-acid content of the urine²³.

Primary vitamin D-resistant rickets is congenital and usually hereditary. It is characterized by lower plasma phosphate levels and an increase in the phosphate excretion index. The vitamin D activity of the serum must be 10-20 times the normal value if the calcium metabolism is to be restored to normal²⁴. This form of rickets may be due to a defect of vitamin D metabolism^{25, 26} and excretion.

Treatment

Prophylaxis of rickets: Sun-baths or quartz lamp treatment, 400 IU vitamin D per day in the pure form or as cod-liver oil.

Treatment of rickets: Rickets and osteomalacia due to a simple deficiency of vitamin D respond to daily oral doses of 3000 IU vitamin D²⁷; in premature infants and children with impaired absorption these should be given intramuscularly. Treatment with massive doses has the disadvantage that there is uncertainty as to the extent to which single high doses are absorbed. High vitamin D doses are called for in primary vitamin D-resistant rickets, and treatment should begin with 50 000 IU per day²⁸. Complete disappearance of the radiological and biochemical symptoms has been reported with a total of 5-400 million IU, depending on the individual²⁴. The maintenance dose, again depending on the individual, ranges from 1000 to 500 000 IU per day²⁴. If growth of the long bones is to proceed normally, treatment must be started immediately after birth²⁷.

Treatment of lupus vulgaris with vitamin D in very massive dosage is now usual only if other tuberculostatic drugs cannot be given.

Toxicology

All the D vitamins are toxic in large quantities. High doses of vitamin D mobilize the bound calcium of the skeleton and bring about a considerable increase in the plasma calcium level as well as in the urinary excretion of phosphate and calcium. The calcium mobilized from the bones is taken up in the soft tissues, particularly the kidneys and media of the vessels. The clinical symptoms are loss of appetite, gastro-intestinal disturbances, pain in the head and joints and muscular weakness; in children other signs are a dry, loose skin, tremor of the limbs, loss of muscle tone with fibrillary spasms and arterial hypertension. When death occurs, this is usually due to renal failure. The symptoms of vitamin D poisoning are reversible when ingestion of the vitamin is stopped.

The toxic effect of vitamin D appears when daily doses exceed 1000-3000 IU per kg body weight and when these doses are given over several months; in infants, hypercalcaemia may appear even with total daily doses of 3000-4000 IU²¹. The clinical appearance of this idiopathic hypercalcaemia of infancy is very similar to that of vitamin D poisoning²⁴. For this reason, infants and pregnant women (since idiopathic hypercalcaemia may already occur in utero) should not be given more than 400 IU vitamin D per day^{21, 29}. Since enrichment of milk, margarine and baby foods with vitamin D is now quite common in many countries, the intake of this substance by infants and children is often excessive, for instance in the USA and Canada up to 2000 IU per day²¹, in England up to 1200 IU per day³⁰.

Vitamin D₂ is less suitable for treating rickets since its hypercalcaemic effect at high dosage is greater than that of vitamin D₃. Although dihydrotachysterol has only a very slight antirachitic activity it is, like vitamin D, capable of increasing the serum calcium level. The danger of poisoning is the same, however, with both substances. In the treatment of hypercalcaemia it is essential that the serum calcium level should be watched.

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Vitamin E¹⁻³ (for references see page 467)

Chemistry⁴

The tocopherols are viscous, yellowish oils readily soluble in organic solvents and insoluble in water. They are stable to acids, alkalis and heat, unstable to oxidizing agents, particularly when exposed to light. The ubiquinones and ubiquinonols form yellow crystals. For structure and properties of vitamin E and related compounds see the table on pages 465-466.

Assay

Biological. Antisterility test on female rats⁷; dialuric acid haemolysis test on rats⁸.

Chemical⁹. Using the reducing properties of the tocopherols, for instance reduction of ferric chloride (EMMERICH-ENGEL reaction). When biological materials require to be assayed, prior separation of other reducing substances is necessary, for instance by molecular distillation or column chromatography. The individual tocopherols can be separated by column, thin-layer, paper or gas chromatography.

Unit

By weight. Formerly 1 International Unit (IU) = 1 mg dl- α -tocopherol acetate = 1 International Rat Unit = the amount required to be given orally to tocopherol-deficient rats to prevent absorption of the foetus in 50%¹⁰.

1 IU = 1.00 mg dl- α -tocopherol acetate = 1.10 mg dl- α -tocopherol = 0.73 mg d- α -tocopherol acetate = 0.81 mg d- α -tocopherol.

Biogenesis

In young plants most of the vitamin E synthesized consists of α -tocopherol, whereas in seeds the other tocopherols predominate¹¹. The individual steps of biogenesis are unknown but it is possible

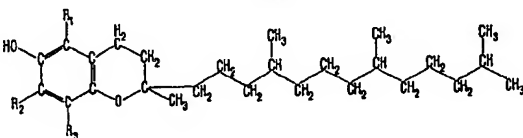
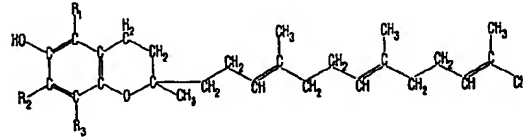
(continued on page 466)

Structure and properties of vitamin E and r

Names*	Formula and mol. wt.	
Tocol	C ₅₆ H ₁₀₄ O ₈ 388.64	R ₁
8-Methyltolcol (8-tocopherol)	C ₅₇ H ₁₀₆ O ₈ 402.67	R ₁
5,8-Dimethyltolcol (3-tocopherol)	C ₅₈ H ₁₀₈ O ₈ 416.69	R ₁
7,8-Dimethyltolcol (γ -tocopherol)	C ₅₈ H ₁₀₈ O ₈ 416.69	R ₁
5,7,8-Trimethyltolcol (α -tocopherol)	C ₅₉ H ₁₁₀ O ₈ 430.72	R ₁
8-Methyltocotrienol (8-tocotrienol)	C ₅₇ H ₁₀₂ O ₈ 396.62	R
5,8-Dimethyltocotrienol (ϵ -tocopherol, β -tocotrienol)	C ₅₈ H ₁₀₄ O ₈ 410.65	R
7,8-Dimethyltocotrienol (γ -tocotrienol)	C ₅₈ H ₁₀₄ O ₈ 410.65	P
5,7,8-Trimethyltocotrienol (ζ -tocopherol, α -tocotrienol, tocochromanol-3)	C ₅₉ H ₁₀₆ O ₈ 424.67	F

* Trivial names recommended by the International Union of Biochemistry (*Biochim.*)
** Relative activity in antisterility test on

Structure and properties of vitamin E and related compounds

Names*	Formula and mol. wt.	Structure	Main source	Activity**
<p style="text-align: center;"><i>Tocols</i></p> 				
Tocol	$C_{55}H_{104}O_2$ 388.64	$R_1 = H, R_2 = H, R_3 = H$	Synthetic	Inactive
8-Methyltol (8-tocopherol)	$C_{57}H_{106}O_2$ 402.67	$R_1 = H, R_2 = H, R_3 = CH_3$	Soybean oil	1
5,8-Dimethyltol (8-tocopherol)	$C_{59}H_{108}O_2$ 416.69	$R_1 = CH_3, R_2 = H, R_3 = CH_3$	Wheat-germ oil	33
7,8-Dimethyltol (γ-tocopherol)	$C_{59}H_{108}O_2$ 416.69	$R_1 = H, R_2 = CH_3, R_3 = CH_3$	Maize-germ oil	10
5,7,8-Trimethyl- tol (α-tocopherol)	$C_{59}H_{108}O_2$ 430.72	$R_1 = CH_3, R_2 = CH_3, R_3 = CH_3$	Maize-germ oil, wheat-germ oil, etc., animal tissues	100
<p style="text-align: center;"><i>Tocotrienols</i></p> 				
8-Methyltoco- trienol (8-tocotrienol)	$C_{57}H_{100}O_2$ 396.62	$R_1 = H, R_2 = H, R_3 = CH_3$	Palm oil	
5,8-Dimethyltoco- trienol (α-tocopherol, β-tocotrienol)	$C_{59}H_{102}O_2$ 410.65	$R_1 = CH_3, R_2 = H, R_3 = CH_3$	Wheat	5
7,8-Dimethyltoco- trienol (γ-tocotrienol)	$C_{59}H_{102}O_2$ 410.65	$R_1 = H, R_2 = CH_3, R_3 = CH_3$	Rice	
5,7,8-Trimethyl- tocotrienol (δ-tocopherol, α-tocotrienol, tocochromanol-3)	$C_{59}H_{102}O_2$ 424.67	$R_1 = CH_3, R_2 = CH_3, R_3 = CH_3$	Wheat	30

* Trivial names recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry (*Biochim. biophys. Acta (Amst.)*, 107, 1 and 5 (1965)).
 ** Relative activity in antisterility test on rats.

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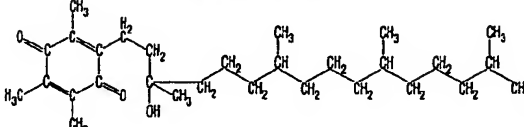
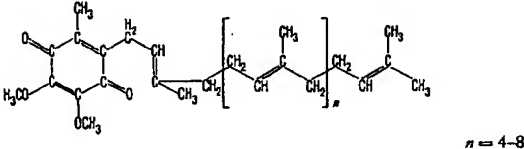
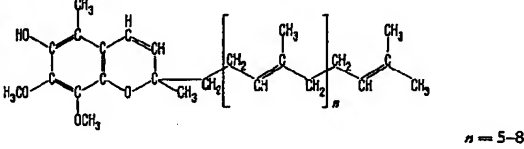
ing properties of the tocopherols, for
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ernational Unit (IU) = 1 mg *d*-α-toco-
 onal Rat Unit = the amount required
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pherol acetate = 1.10 mg *d*-α-toco-
 rol acetate = 0.81 mg *d*-α-tocopherol.

c vitamin E synthesized consists of α-
 the other tocopherols predominate¹¹.
 enesis are unknown but it is possible
 (continued on page 466)

Structure and properties of vitamin E and related compounds (continued)

Names*	Formula and mol. wt.	Structure	Main source	Activity**
Tocopherol-like compounds				
α -Tocopherylquinone (α -tocopherol-quinone)	$C_{55}H_{100}O_2$ 446.72		Oxidation product of α -tocopherol, green plants	Active ⁶
Ubiquinones (coenzymes Q)		 $n = 4-8$	Ubiquinone-9 (ubiquinone-45, coenzyme Q ₉)*: leaves; ubiquinone-10 (ubiquinone-50, coenzyme Q ₁₀)*: liver, yeast	
Ubichromenols		 $n = 5-8$	As corresponding ubiquinones	Active ⁶
* Trivial names recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry [<i>Biochim. biophys. Acta (Amst.)</i> , 107, 1 and 5 (1965)]. ** Relative activity in antistilluric test on rats.				

that the process resembles the synthesis of the ubiquinones (formation of the terpenoid chain of mevalonic acid and of the aromatic ring from phenylalanine)¹².

Intake and excretion

The daily intake of adults in the USA has been estimated at 24 mg total tocopherols and 14 mg α -tocopherol¹², by another authority at 7.4 mg α -tocopherol¹⁴. The tocopherol esters are hydrolysed in the small intestine, and bile is necessary for their absorption. Probably only about 35% of the tocopherols in food is absorbed, the remainder being excreted in the faeces¹⁵. The normal concentration in adult serum is ca. 10 mg/l, in newborn infants ca. 5 mg/l (see page 609). The maximum blood level is reached 4-9 hours after giving tocopherols^{16, 17}.

α -Tocopherol is stored in the liver and fatty tissues. High concentrations occur in the pituitary, adrenals, uterus and testes¹⁸. In the liver, tocopherol has been found in the mitochondria and microsomes¹⁹. The amount of tocopherols stored by the body is several grammes². In the fatty tissues this is believed to include also α -tocopherylquinone, an oxidation product of α -tocopherol¹⁹.

The metabolites tocopheronic acid and tocopheronolactone have been isolated in the form of glucuronides (SIMON metabolite)²⁰ from the urine of persons given large amounts of tocopherol.

Function

The tocopherols act as antioxidants in the following processes both in vitro and in vivo^{21, 22}:

- they prevent the oxidation of unsaturated fatty acids (linoleic acid) to peroxides; lipoperoxides are associated with the formation of the yellowish brown pigment in smooth muscle (ceroid pigment)
- they prevent the oxidation of vitamin A and the carotenes
- they prevent the oxidation of thiol groups, particularly in enzymes, presumably in conjunction with selenium²³.

The relationship between vitamin E and cholesterol metabolism is obscure²². The tocopherols may play a role in nucleic acid metabolism²⁴ and in erythropoiesis²⁵. There may be a functional con-

nection between the tocopherols and the ubiquinones, which are involved in electron transport²⁶, and the formation of adenosine triphosphate (see page 403)²⁷. Vitamin E seems to have an effect on the transport and metabolism of vitamin B₁₂²⁸.

Requirements and deficiency symptoms

Healthy adults require 10-30 mg α -tocopherol per day²⁹ (see also page 494), depending on the intake of polyene fatty acids³⁰, 0.6 mg α -tocopherol at least being required per gramme polyene fatty acid³¹. The minimal requirement of infants is probably 0.5 mg per kg body weight³², an amount normally absorbed with the breast milk.

Vegetable oils are rich in tocopherols, especially wheat-germ oil with 200-300 mg/100 g. Other good sources of tocopherols are cereals and eggs. Animal tissues contain little tocopherol, mainly as α -tocopherol.

Vitamin E deficiency results in various changes depending on the species, age and nutritional state. They include impaired reproductivity and absorption of the foetus (rats, mice, guinea-pigs), muscular dystrophy accompanied sometimes by marked creatinuria (monkeys, mice, etc.), formation of ceroid pigments (monkeys, mice, swine), increased haemolysis in vitro (rats, chickens), encephalomalacia (chickens), exudative diathesis (chickens), necrosis of the liver ('respiratory decline' in rats), renal autolysis (rats).

In human beings the signs of vitamin E deficiency are not very marked.

A measure of the vitamin E status is provided by the peroxide haemolysis test³³. In many persons a lowered serum tocopherol level is associated with an increase of in vitro haemolysis³⁰. A low serum level is common in newborn infants and particularly premature infants, and vitamin E deficiency is a possible cause of macrocytic anaemia³⁴ and haemolytic anaemia³⁵ in infants. Vitamin E deficiency may also be caused by impaired absorption of fats^{3, 36}. Thus low tocopherol serum levels, often combined with creatinuria and the deposition of ceroid pigments in the smooth muscle (gastro-intestinal tract), have been observed in sprue, coeliac disease, biliary cirrhosis, pancreatitis and particularly cystic fibrosis of the pancreas. A definite relationship between muscular dystrophy and vitamin E

deficiency has not been proved³⁷. A few of the fatty tissues of premature infants > neonous connective tissue has been reported.

Treatment³⁸

Administration of tocopherols is indicated, in disturbances of fat absorption of the pancreas), in premature infants and in persons whose diet includes large fatty acids. In cystic fibrosis of the pancreas should be given daily³⁷. Inclusion of women before childbirth is considered on the capillary resistance of the infant appear to be toxic even in large doses.

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Vitamin K^{1, 2}

Chemistry

The K vitamins are soluble in fats, > stable to light. Solubility in water is con bialphite and the tetrasodium salt of hyd

deficiency has not been proved³⁷. A very low vitamin E content in the fatty tissues of premature infants with oedema of the subcutaneous connective tissue has been reported³⁸.

Treatment³⁹

Administration of tocopherols is indicated in intermittent claudication, in disturbances of fat absorption (particularly cystic fibrosis of the pancreas), in premature infants nurtured on cow's milk and in persons whose diet includes large amounts of unsaturated fatty acids. In cystic fibrosis of the pancreas at least 100 mg α -tocopherol should be given daily¹⁷. Inclusion of vitamin E in the diet of women before childbirth is considered to have a favourable effect on the capillary resistance of the infant⁴⁰. The tocopherols do not appear to be toxic even in large doses.

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Vitamin K^{1,2}

Chemistry

The K vitamins are soluble in fats, fairly stable to heat but unstable to light. Solubility in water is confined to menadione sodium bisulphate and the tetrasodium salt of hydrovitamin K₁ diphosphate.

For structure and properties of vitamin K and related compounds see the table on page 468.

Assay

Biological. By curative effect on vitamin K-deficient chicks².

Chemical³. In pure solution spectrophotometrically, fluorimetrically, colorimetrically, polarographically, also oxidimetrically after reduction to the hydroquinone.

Assay of the vitamin in animal or vegetable material must be done chromatographically or after a suitable extraction process.

Unit

No international unit; by weight. The following units are occasionally used: 20 DAM units = 1 ANSACHER unit = 0.0008 mg menadione = the minimum amount required to normalize the prothrombin time within 6 hours in vitamin K-deficient chicks weighing 70-100 g.

Biogenesis

Vitamin K₁ is synthesized in green plants under the effect of light and accumulates mainly in the chloroplasts. The K₂ vitamins are synthesized by certain intestinal bacteria. It is not known how the naphthoquinone ring is formed.

Intake and excretion

Bile or bile acids are necessary for optimal absorption of vitamin K, both from foods and from the vitamin synthesized by the intestinal flora; menadione and the synthetic water-soluble preparations are absorbed without the intervention of bile. Vitamin K is transported by the lymph. According to MARTIUS⁴, vitamin K of vegetable or bacterial origin undergoes a conversion in the animal organism in which the various side chains are replaced uniformly by an isoprenoid chain of 20 C-atoms. The original side chains are split off by the action of intestinal bacteria with the formation of methyl-naphthoquinone. The latter is absorbed and converted in animal tissues into the specific vitamin K_{2(10)}} of the animal organism by introduction of the geranylgeranyl chain.

Vitamin K is not stored in the tissues when ingested in quantities comparable with those in the diet. When large doses are given, the vitamin accumulates in the liver and spleen⁵. After administration of radioactive vitamin K to rats, part of the activity has been found in the urine and bile⁶. Metabolites of the vitamin have been isolated from the bile of dogs⁷.

Function

Vitamin K is involved in the blood coagulation mechanism and is responsible for the maintenance of a normal prothrombin time. It also affects the formation of prothrombin (factor II), factor VII, factor IX, factor X and perhaps also factor V⁸. Here the vitamin possibly acts by promoting the formation of the quaternary protein structure (S-S bridges)⁹; it may also be involved directly in prothrombin synthesis¹⁰. Whether the vitamin plays a role in oxidative phosphorylation is not yet clear¹¹, although such a role has been demonstrated in bacteria¹². In the light of the quinone-hydroquinone structure of vitamin K and its ready conversion into a chromanol, the function of the vitamin is most likely that of an electron carrier⁹.


Dicumarol and related components are vitamin K antagonists in blood coagulation, whence their use as anticoagulants; their anticoagulant effect can be inhibited by vitamin K.

Requirements and deficiency symptoms

The human requirement of vitamin K is unknown, but ample amounts appear to be available, except in newborn infants, from a normal diet combined with synthesis by the intestinal bacteria, though the latter does not seem to play any great role in man¹³. Tests have shown that in newborn infants 5 µg disodium 2-methyl-1,4-naphthohydroquinone disuccinate per day are adequate to ensure maximum prothrombin activity¹⁴.

Vitamin K is found mainly in green vegetables such as spinach and cabbage; the amounts in tomatoes and liver are less, and very little is present in fruits, milk and meat. Cow's milk contains more of the vitamin than breast milk.

Vitamin K deficiency causes a hypoprothrombinaemia marked by an excessive prothrombin time and a tendency to bleeding. A reduced prothrombin activity due to vitamin K deficiency occurs

	Main source	Activity ²⁰
	Oxidation product of α -tocopherol, green plants	Active ⁵
$\gamma = 4-8$	Ubiquinone-9 (ubiquinone-45, coenzyme Q ₉) ¹⁵ : leaves; ubiquinone-10 (ubiquinone-50, coenzyme Q ₁₀) ¹⁶ : liver, yeast	
$\gamma = 5-8$	As corresponding ubiquinones	Active ⁶

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herols and the ubiquinones, which are not²⁶, and the formation of adenosine²⁷. Vitamin E seems to have an effect on the formation of B₁₂²⁸.

Deficiency symptoms

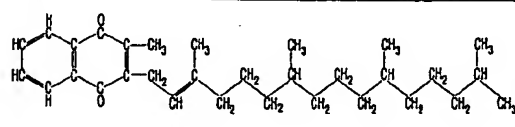
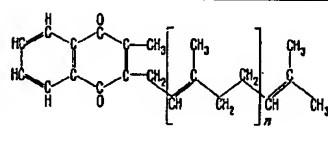
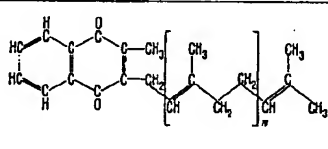
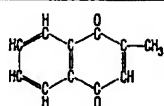
0-30 mg α -tocopherol per day²⁹ (see on the intake of polyene fatty acids³⁰, it being required per gramme polyene requirement of infants is probably 0.5 mg amount normally absorbed with the breast

tocopherols, especially wheat-germ oil. Other good sources of tocopherols are: various contain little tocopherol, mainly

its in various changes depending on the state. They include impaired reproduction: foetus (rats, mice, guinea-pigs), muscled sometimes by marked creatinuria, necrosis of ceroid pigments (monkeys), myolysis in vitro (rats, chickens), excretory diathesis (chickens), necrosis cline³¹ in rats, renal atrophy (rats). As of vitamin E deficiency are not very

in E status is provided by the peroxide: persons a lowered serum tocopherol increase of in vitro haemolysis³². A low newborn infants and particularly prematurity deficiency is a possible cause of macrocytic anaemia³³ in infants. Vitamin E ed by impaired absorption of fats³⁴, levels, often combined with creatinuria pigments in the smooth muscle (gastro-intestinal sprue, coeliac disease, biliary particularly cystic fibrosis of the pancreas. When muscular dystrophy and vitamin E

Structure and properties of vitamin K and related compounds

Names*	Formula and mol. wt.	Structure	Physical properties	Occurrence	Activity **
Vitamin K ₁ (₉₀) (phyloquinone*)	C ₃₁ H ₄₆ O ₂ 450.71		Yellow oil M.p. -20 °C	Green plants, tomatoes, some bacteria. Isolated from alfalfa	100
Vitamin K ₂ (₉₀) (menaquinone-6*)	C ₄₁ H ₅₄ O ₂ 580.90	 n = 5	Yellow crystals M.p. 50 °C	Isolated from putrid fishmeal	100
Vitamin K ₃ (₉₃) (menaquinone-7*)	C ₄₃ H ₅₆ O ₂ 649.02	 n = 6	Yellow crystals M.p. 54 °C	Some bacteria. Isolated from putrid fishmeal	70
Menadione (vitamin K ₃ , methyl-naphthoquinone)	C ₁₁ H ₈ O ₂ 172.19		Yellow needles M.p. 106 °C	Synthetic. Possibly a metabolite	ca. 100

* Trivial names recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry [*Biochim. biophys. Acta (Amst.)*, 107, 5 (1965)].

** Relative activity in vitamin K-deficient chicks.

in some intestinal complaints like severe diarrhoea¹⁸ and steatorrhoea¹⁹, as well as in impaired absorption of the vitamin due to lack of bile (biliary fistula, obstruction of the bile ducts). The hypoprothrombinemia associated with severe injury to the liver parenchyma is not due, however, to vitamin K deficiency and is also not reversed by administration of the vitamin; this forms the basis of a test of liver function. Vitamin K deficiency can also occur during long-term treatment with antibiotics or sulphonamides as a result of destruction of the intestinal flora.

During the first days of life the prothrombin activity of the plasma is 10–50% of that in adults¹⁷, possibly because the intestinal flora is not sufficiently developed and intake of the vitamin with milk is small. Among newborn infants not given vitamin K, 0.1–1% suffer from bleeding, a proportion that has been shown to be reducible by vitamin K treatment^{18,19}.

Treatment

Vitamin K is given prophylactically to newborn infants, particularly premature infants and those with anoxia, the dosage of the vitamin (or of a water-soluble preparation) being 0.5–1 mg subcutaneously or intramuscularly or 1–2 mg orally at birth¹⁸. Double these doses may be necessary in children whose mothers have been treated with anticoagulants. Administration of the vitamin to mothers ante partum is not recommended²⁰. The prophylactic use of the water-soluble menadione derivatives in pregnant women and newborn children is inadvisable on account of the danger of hyperbilirubinaemia and an increased tendency to kernicterus; these effects may be due not to the menadione derivatives themselves but to their intermediary metabolites.

The vitamin is given to correct low prothrombin activity due to a deficiency (see under 'Requirements and deficiency symptoms', above) as well as to overdosage of anticoagulants.

If possible, vitamin K should be given by the oral, intramuscular or subcutaneous route and not intravenously²¹.

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Thiamine¹⁻³

Structure and properties of thiamine and r

Compound	Formula and mol. wt.
Thiamine (vitamin B ₁ , aneurin)	C ₁₂ H ₁₇ N ₄ OS (cation) 265.36 C ₁₂ H ₁₆ N ₄ OSCl ₂ (hydrochloride) 337.27
Thiamine diphosphate (TDP) Thiamine pyrophosphate (TPP) (cocarboxyl- ase)	C ₁₂ H ₁₈ N ₄ O ₇ P ₂ S 424.31
α-Hydroxy- ethyl-2-thia- mine diphos- phate α-Hydroxy- ethyl-2-thia- mine pyro- phosphate	C ₁₁ H ₂₂ N ₄ O ₇ P ₂ S 468.37
Thiochrome	C ₁₂ H ₁₄ N ₄ OS 262.34
Oxythiamine	C ₁₂ H ₁₆ N ₄ O ₃ S 266.34

Assay

Biological. Rat protection test, rat gr. **Microbiological.** Thiamine with *Ochro monas danica*⁴; pyrimidine and thiazole derivatives⁵.

Enzymatic. With apodecarboxylase f **Chemical**⁶. Quantitatively in pure : chloride, gravimetrically as reineckate, derivatives from thiamine and diazo, with cyanogen bromide; in biological i mine to thiochrome, which shows stroi violet light; with suitable modification: be used to determine the mono-, di- ar as well as the protein-bound thiamine.

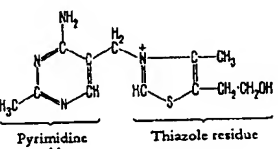
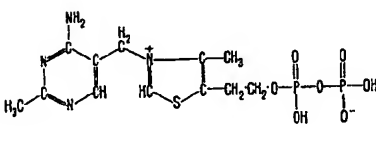
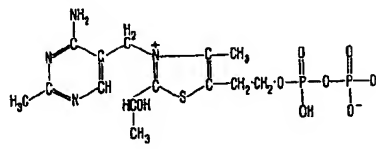
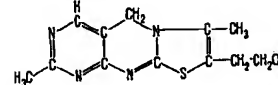
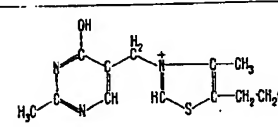
Thiamine

(For references see page 471)

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Thiamine¹⁻³

Structure and properties of thiamine and related compounds

Compound	Formula and mol. wt.	Structure	Physical properties	Occurrence and activity
Thiamine (vitamin B ₁ , aneurin)	C ₁₂ H ₁₇ N ₄ OS (cation) 265.36 C ₁₂ H ₁₈ N ₄ OSCl ₂ (hydrochloride) 337.27		Colourless needles, readily soluble in water, odourless when pure, thermolabile in neutral and alkaline solution, stable to atmospheric oxygen, unstable to oxidizing agents and ultraviolet light. M.p. 245-248 °C (hydrochloride)	Plants. In animal tissues as thiamine pyrophosphate
Thiamine diphosphate (TDP) Thiamine pyrophosphate (TPP) (cocarboxylase)	C ₁₈ H ₂₅ N ₄ O ₇ P ₂ S 424.31		Pale yellow needles, readily soluble in water. M.p. 242-244 °C	Animal tissues. Cofactor of decarboxylases and other enzymes
α-Hydroxyethyl-2-thiamine diphosphate α-Hydroxyethyl-2-thiamine pyrophosphate	C ₁₄ H ₂₃ N ₄ O ₈ P ₂ S 468.37			Micro-organisms; represents 60% of the total thiamine in <i>E. coli</i> ⁴ . Also known as 'active acetaldehyde' (see page 391)
Thiochrome	C ₁₁ H ₁₄ N ₄ OS 262.34		Yellow prisms showing blue fluorescence in solution. M.p. 277-278 °C	Oxidation product of thiamine
Oxythiamine	C ₁₂ H ₁₄ N ₄ O ₂ S 266.34		M.p. 195-200 °C	Antagonist of thiamine

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should be given by the oral, intramuscular or not intravenously²¹.

1. *The Vitamins*, vol. 2, Academic Press, New York and Wts., *Vitam. and Horm.*, 17, 53 (1959); WOHL and GOODHART (Eds.), *Modern Nutrition* 1., Lea & Febiger, Philadelphia, 1964, page 367. BEATON and MCHENRY (Eds.), *Nutrition*, vol. 2, 1964, page 1. *Praktische Vitaminbestimmungsmethoden*, 5th ed., 400. *Vitshr.*, 93, 1264 (1963). (Kb.), 10, 58 (1954) and 11, 90 (1955). *Vitshr.*, 84, 792 (1954); TAYLOR et al., *Canad. J. Sci.*, 107, 123 (1965). *Child.*, 97, 524 (1959). 22, 225 (1964). 926 (1964); HILL et al., *J. biol. Chem.*, 243, 3930

Vitshr., 83, 1701 (1958). 3, 995 (1961). *Acta*, 194, 127 (1965). *Prothrombinaktivitäten bei Myofibrillen mit en. Analyse* (Thesis), Munksgaard, Copenhagen.

Child., 80, 944 (1950). 7 (1960). *Acta*, 15, 279 (1954); McELKESH, A. E., *Amer.*

iatrics, Committee on Nutrition, *Pediatrics*, 28, 343 (1955); WEFRING, K. W., *J. Pediatr.*, 61, 686

Assay

Biological. Rat protection test, rat growth test; now little used. **Microbiological.** Thiamine with *Ochromonas malhamensis*⁵ or *Ochromonas danica*⁶; pyrimidine and thiazole residues with *Saccharomyces cerevisiae*⁷.

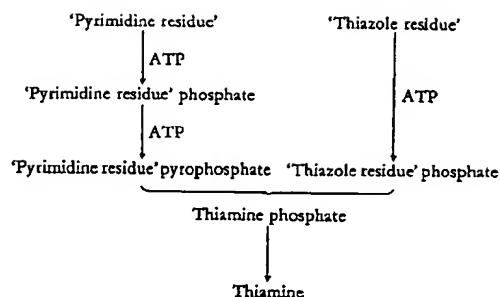
Enzymatic. With apodecarboxylase from yeast⁸. **Chemical**⁹. Quantitatively in pure solution by titration of the chloride, gravimetrically as reineckate, colorimetrically with the azo derivatives from thiamine and diazonium salts, fluorimetrically with cyanogen bromide; in biological material by oxidation of thiamine to thiochrome, which shows strong blue fluorescence in ultraviolet light; with suitable modifications the thiochrome method can be used to determine the mono-, di- and triphosphates of thiamine as well as the protein-bound thiamine.

Units

No international unit; by weight. The former International Unit (= 0.003 mg thiamine hydrochloride = the U.S. Pharmacopoeia Unit) is now obsolete.

Biogenesis¹⁰

Thiamine is synthesized by plants, many bacteria and algae, and by some fungi. Some micro-organisms can synthesize only the pyrimidine and/or thiazole residues, which are formed independently of one another in a manner largely unknown and then combine to form thiamine. The first product is thiamine monophosphate, which is then hydrolysed to thiamine.



Thiamine, but not thiamine phosphate, is converted by ATP into thiamine pyrophosphate (for instance in yeast and intestinal tissue). ATP can also convert thiamine pyrophosphate into thiamine triphosphate (yeast). On the activation of aldehydes by thiamine pyrophosphate see under 'Function', below.

Intake and excretion

In the USA, the daily diet contains about 2.15 mg thiamine¹¹, in Germany about 1.8 mg¹². Thiamine is readily absorbed in the small intestine and converted enzymatically in the intestinal mucosa into thiamine pyrophosphate. In rats, thiamine is synthesized by the intestinal flora¹³, but in man it is unlikely that bacterial synthesis plays an important role.

In whole blood the thiamine content is 20–75 µg/l, in serum 18–62 µg/l, in spinal fluid 3–12 µg/l¹⁴. Erythrocytes contain 80 µg/l, leucocytes 675 µg/l¹⁴. The thiamine content of the serum in newborn children is very high (see page 609). Small amounts of free thiamine are present in the serum, whereas in the erythrocytes and tissues the main component is thiamine pyrophosphate. The presence of thiamine monophosphate and triphosphate and thiochrome in the tissues has often been reported¹⁵. The heart muscle is fairly rich in thiamine (2–3 µg/g), as are the brain, liver and kidneys (1 µg/g); smaller quantities are present in the skeletal muscles (0.5 µg/g)¹⁶. The human liver contains about 4 mg of thiamine¹⁷.

When the daily dietary intake of thiamine rises above 0.5–0.6 mg the urinary excretion of the vitamin increases in proportion to the intake; on an ample diet it amounts to at least 100 µg/day^{2,18} (see also page 676). The urine also contains breakdown products (pyrimidine and thiazole residues) the amounts of which are not proportional to the thiamine content of the diet, so that they are regarded as a measure of the rate at which body stores of thiamine are being depleted¹⁹. The thiamine content of breast milk (see page 689) depends on the thiamine intake and shows large individual variations.

Function

Thiamine pyrophosphate possesses coenzyme functions in the breakdown of carbohydrates (oxidative decarboxylation of pyruvate, see page 391), in the citric acid cycle (oxidative decarboxylation of α-ketoglutarate, see page 390), in the pentose phosphate cycle (transketolase reaction, see page 421) and other biochemical reactions²⁰; at least 24 enzymes are known that contain thiamine pyro-

Reaction	Active aldehyde
Oxidative decarboxylation of pyruvate	Active pyruvate (α-lactyl-2-thiamine pyrophosphate) ²¹ Active acetaldehyde (α-hydroxyethyl-2-thiamine pyrophosphate) ²¹
Oxidative decarboxylation of α-ketoglutarate	Active α-ketoglutarate (?) Active succinate semialdehyde (?)
Transketolase reaction	Active xylulose 5-phosphate (?) Active glyceraldehyde ²¹ Active sedoheptulose 7-phosphate (?)
Glyoxylate carboxylase reaction	Active glyoxylate ²² Active formaldehyde ²² Active tartronic semialdehyde ²²

phosphate as coenzyme². The active aldehyde group in these reactions is formed or transported by thiamine pyrophosphate enzymes and is bound to the C-2 atom of the thiazole ring (see table).

Thiamine pyrophosphate plays an important part in the production of stimuli in the peripheral nerves and in the recovery process after stimulation²³; during stimulation of the peripheral nerves thiamine is liberated from thiamine pyrophosphate²⁴.

The thiamine-sparing effect of dietary fats probably derives from the fact that in thiamine deficiency the activity of pyruvate dehydrogenase is more rapidly inhibited than that of oxoglutarate dehydrogenase (α-ketoglutarate dehydrogenase)²; in thiamine deficiency, however, toxic products may also be formed from carbohydrates²⁰.

Various synthetic compounds resembling thiamine in structure, such as oxythiamine, pyriothiamine and neopyriothiamine, act as thiamine antagonists²⁵; antithiamine factors of unknown structure occur in bacteria, plants and animals (particularly in cold-blooded animals, where the antimetabolite concerned is also known as 'thiaminase')²⁶.

Requirements and deficiency symptoms

The requirement of thiamine depends primarily on the intake of carbohydrates, although in practice it is usually related to the calorie intake. In adults the minimum requirement is about 0.27–0.33 mg per 1000 kcal²⁰. In bottle-fed infants the maintenance dose has been given as 0.14–0.20 mg per day²⁰. The Joint FAO/WHO Expert Group²⁷ recommends a daily thiamine intake of 0.4 mg per 1000 kcal for infants, children, adults and pregnant and lactating women (see page 493). The allowances of the Food and Nutrition Board (USA)²⁸ (see page 494) are based on a daily intake of 0.5 mg thiamine per 1000 kcal. The requirement of thiamine increases with the metabolic rate. The possible presence of antithiamine factors in the diet must also be allowed for.

Good sources of thiamine are yeast, pork, liver, kidneys and wholemeal cereals (see pages 507–508). The vitamin is partly destroyed during cooking, particularly in alkaline media, but is unaffected by deep freezing¹.

The classical symptoms of vitamin B₁ deficiency are anorexia, nausea and vomiting. Other symptoms are fatigue, weakness, hypotonia of the gastrointestinal tract and disturbances of the peripheral nerves (weakness in the limbs, hyperaesthesia and paraesthesia, disturbances of coordination). Emotional disturbances are also observed, such as depression, irritability and impairment of memory and power of concentration.

Beriberi takes various forms according to the predominating symptoms:

(a) An exudative form, in which oedema is the first symptom. This may be followed by enlargement of the heart and right-sided heart failure with sudden death.

(b) A 'dry' form in which the main symptoms are polyneuritis of peripheral degenerative type and atrophy of the muscles of the limbs. In European latitudes thiamine deficiency is marked mainly by polyneuritis; it is seen for example in chronic alcoholism, although this condition is probably accompanied in general by deficiency of several of the B vitamins³⁰.

(c) A rare cerebral form with the symptoms of WERNICKE's disease, namely nystagmus, ocular paralysis and emotional disturbances (irritability, sleeplessness, loss of memory, disorientation, confabulation, hallucinations), followed by loss of consciousness and death. This disease is seen for instance in Europe among chronic alcoholics and occasionally in patients with cancer.

(d) An infantile form seen in the first year of life and a principal cause of the high infant mortality in southern and southeastern Asia; thus between 1954 and 1958 in the Philippines 15 000 children died each year from beriberi³¹. The chronic form is manifested by a slow growth rate, constipation, vomiting and oedema, the acute form by heart failure and death. Occasionally the symptoms resemble those of meningitis or encephalitis. The cause of this vitamin deficiency disease is still to some extent obscure. In most cases the mother suffers from thiamine deficiency so that the maternal milk is deficient in this vitamin; toxic substances in the milk may also play a role³².

Biochemically, thiamine deficiency is characterized by a low urinary concentration of the vitamin (in beriberi 0–14 µg per 24 h), by disturbances of carbohydrate metabolism (increased blood pyruvate and α-ketoglutarate levels³³) and by a low tissue concentration of thiamine pyrophosphate (erythrocytes³⁴, brain³⁵). The thiamine pyrophosphate content of the erythrocytes can be measured by means of the transketolase activity, which in thiamine deficiency can be normalized by administering thiamine pyrophosphate³⁴. The biochemical changes precede the pathological symptoms; it

is not known, however, how the lesion of the heart and in the nervous system arises to the tissues is too low, or there may be metabolites.

Order in which thiamine deficiency symptoms appear (per day)³⁴

Days	Biochemical symptoms
5	Urinary thiamine 50 µg/24 h
10	Urinary thiamine 25 µg/24 h, transketolase activity of erythrocytes somewhat low
21–28	Urinary thiamine 0–25 µg/24 h, transketolase activity of erythrocytes 15–25% lower
30–200	Urinary thiamine negligible, transketolase activity of erythrocytes 35% lower
over 200	Urinary thiamine negligible, transketolase activity of erythrocytes over 45% lower

Treatment

In adults beriberi is usually treated with a dosage of 20–30 mg thiamine per day; usually successful, but when the heart is involved should be given initially by the parenteral administration results in: chemical changes usually regress rapidly, but there is occasional irreversible Thiamine has been used in the treatment of its successful application deficiency.

Since thiamine hydrochloride is only slightly decomposed in alkaline media, but with fewer disadvantages³⁶, for instance phosgene, have been synthesized.

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active aldehyde group in these react by thiamine pyrophosphate enzymes of the thiazole ring (see table), plays an important part in the production of nerves and in the recovery process stimulation of the peripheral nerves thiamine pyrophosphate²⁴. of dietary fats probably derives from deficiency the activity of pyruvate dehydrogenase²⁵; in thiamine deficiency, also be formed from carbohydrates²⁶, and resembling thiamine in structure, thiamine and neopyrithiamine, act as thiamine factors of unknown structure animals (particularly in cold-blooded animals) concerned is also known as

Deficiency symptoms

Deficiency depends primarily on the intake of thiamine. It is usually related to the caloric requirement is about 0.27-0.33 mg in infants the maintenance dose has been 1 mg²⁷. The Joint FAO/WHO Expert Group thiamine intake of 0.4 mg per 1000 kcal and pregnant and lactating women of the Food and Nutrition Board based on a daily intake of 0.5 mg thiamine of thiamine increases with the presence of antithiamine factors in the diet. These are yeast, pork, liver, kidneys and 507-508). The vitamin is partly deficient in alkaline media, but is un-

deficiency of vitamin B₁ deficiency are incipient. Other symptoms are fatigue, weakness, intestinal tract and disturbances of the heart, hyperaesthesia and paraesthesia. Emotional disturbances are common, irritability and impairment of concentration.

Symptoms according to the predominating

1. which oedema is the first symptom. enlargement of the heart and right-sided heart.

2. the main symptoms are polyneuritis type and atrophy of the muscles of the heart. Thiamine deficiency is marked mainly by example in chronic alcoholism, usually accompanied in general by deficiencies²⁸.

3. with the symptoms of Wernicke's disease: paralysis and emotional disturbances of memory, disorientation, confabulation by loss of consciousness and or instance in Europe among chronic alcohol patients with cancer.

4. in the first year of life and a principal mortality in southern and southeastern 1958 in the Philippines 15 000 children²⁹. The chronic form is manifested by a chronic, vomiting and oedema, the acute form. Occasionally the symptoms resemble encephalitis. The cause of this vitamin deficiency is of extreme importance. In most cases the deficiency is so that the maternal milk contains toxic substances in the milk may also

efficiency is characterized by a low uric acid (in beriberi 0-14 µg per 24 h), by a low metabolism (increased blood pyruvate³⁰) and by a low tissue concentration of erythrocytes³¹, brain³². The thiamine in the erythrocytes can be measured by activity, which in thiamine deficiency is missing thiamine pyrophosphate³³. The deficiency of the pathological symptoms; it

is not known, however, how the lesions in the vascular system of the heart and in the nervous system arise. Possibly the energy supply to the tissues is too low, or there may be an accumulation of toxic metabolites.

Order in which thiamine deficiency symptoms appear (0.2 mg thiamine per day)³⁴

Days	Biochemical symptoms	Clinical symptoms
5	Urinary thiamine 50 µg/24 h	-
10	Urinary thiamine 25 µg/24 h, transketolase activity of erythrocytes somewhat low	-
21-28	Urinary thiamine 0-25 µg/24 h, transketolase activity of erythrocytes 15-25% lower	Loss of weight, sleeplessness, irritability
30-200	Urinary thiamine negligible, transketolase activity of erythrocytes 35% lower	Increasing weakness, loss of weight, polyneuritis, bradycardia, peripheral oedema, enlargement of heart, ophthalmoplegia
over 200	Urinary thiamine negligible, transketolase activity of erythrocytes over 45% lower	Histopathological changes as a result of the biochemical defects

Treatment

In adults beriberi is usually treated by administering thiamine at a dosage of 20-30 mg thiamine per day³⁵. Oral administration is usually successful, but when the heart is severely affected the vitamin should be given initially by the parenteral route. In rare cases parenteral administration results in anaphylactic shock. The biochemical changes usually regress rapidly; the cardiovascular disturbances are normally reversible but recovery from polyneuritis is slow and there is occasional irreversible injury to the nerves. Thiamine has been used in the treatment of many diseases³⁶, but a condition of its successful application is the existence of a thiamine deficiency.

Since thiamine hydrochloride is only partly absorbed and is readily decomposed in alkaline media, biologically active derivatives with fewer disadvantages³⁷, for instance thiamine propyl disulphide, have been synthesized.

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Riboflavin¹⁻² (for references see page 473)

Chemistry

For structure and properties of riboflavin and related compounds see the table on page 472.

Assay

Biological³. Growth test on rats or chicks.
Microbiological⁴. With *Lactobacillus helveticus* (L. casei), *Leuconostoc mesenteroides* or *Tetrahymena pyriformis*.
Chemical⁵. By mass analysis after oxidation with periodate; polarographically, spectrophotometrically in the UV range; by photometric measurement of the colour; by fluorometric measurement of the colour of the original vitamin or after conversion to lumiflavin by irradiation in alkaline solution. In biological materials assay is best carried out by the latter method after suitable pre-treatment. The individual flavins can be separated by paper chromatography, electrophoresis or ion exchange.

Unit

No international unit; by weight. 1 rat unit = 4 µg riboflavin.

Biogenesis⁶

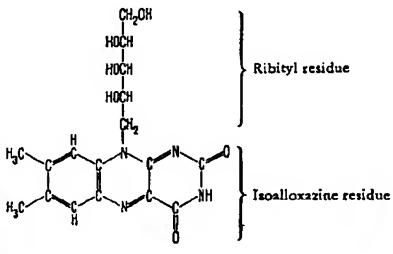
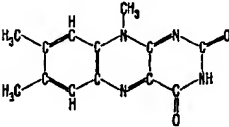
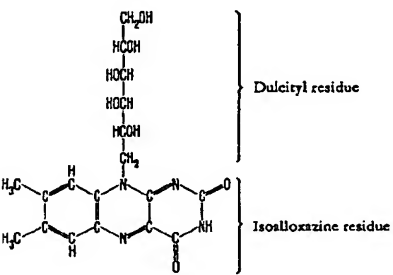
Riboflavin is synthesized by bacteria (such as *Clostridium* spp., *Azotobacter* spp.), by fungi (such as ascomycetes and yeasts) and by plants. Purines, pyrimidines, riboflavin and pteridines are formed in a similar way from glycine, formic acid and carbon dioxide. Probable intermediate products of the biosynthesis are 4,5-diaminouracil and 6,7-dimethyl-8-ribityl-lumazine, so that the pyrimidine ring is the first product. The benzene ring is probably completed by the incorporation of acetate. Riboflavin is possibly synthesized at the FAD stage, with 4,5-diaminouracil adenine dinucleotide as intermediate. The conversion of riboflavin into flavin mononucleotide and flavin adenine dinucleotide is mediated by ATP and also occurs in animal tissues (intestine, liver).

Intake and excretion

In USA the daily diet contains on the average 2.65 mg riboflavin⁷. Riboflavin is absorbed in the small intestine in amounts proportional to the amounts of the vitamin ingested⁸. Riboflavin is also synthesized by the intestinal flora, particularly when the diet contains large amounts of carbohydrates that are not easily digested. A part of the riboflavin so formed may also be absorbed in man⁹. In rats, FAD is absorbed less easily than free riboflavin and FMN¹⁰. Free riboflavin is converted in the intestinal mucosa into FMN, which is then transformed into FAD in the liver¹¹.

The riboflavin content of whole blood¹² is 49-104 µg/l, of serum¹³ 26-37 µg/l, of erythrocytes¹⁴ 180-262 µg/l, of leucocytes¹⁵ 2.27-2.93 mg/l, of liver¹⁶ 25 µg/g, of heart muscle¹⁷ 13 µg/g, of skeletal muscle¹⁸ 2.7 µg/g, of the lens of the eye¹⁹ 3.1 µg/g. In blood (see page 609) and in animal organs 70-90% of the riboflavin is present as FAD, 5-30% as FMN and 0.5-2% as free riboflavin²⁰. In the presence of a positive nitrogen balance²¹ flavoproteins are produced in the tissues (with decreasing urinary riboflavin concentration); in the presence of a negative nitrogen balance²² or during fasting²³ flavoproteins are broken down in the tissues (with increasing urinary riboflavin concentration). The urinary excretion varies with the intake; when the latter is less than 1 mg riboflavin

Structure and properties of riboflavin and related compounds

Names	Formula and mol. wt.	Structure	Physical properties	Occurrence and activity
Riboflavin (vitamin B ₂ , lactoflavin, 7,8-dimethyl- 10-[D-ribityl]- isaloioxazine)	C ₁₇ H ₂₀ N ₄ O ₆ 376.37		Orange-yellow needles, bitter taste, slightly soluble in water and ethanol, readily soluble in acids, stable to heat when dry and to acid media, very unstable to alkalis and light. Yellowish green fluorescence in solution M. p. 275–282 °C (decomp.)	Constituent of flavin mononucleotide and flavin adenine dinucleotide. Occurs in free form in some micro-organisms. Makes up 0.5–2% of total riboflavin of animal organs ⁴ . Present in urine
Flavinmononucleotide (FMN) (riboflavin-5'-phosphate)	See page 345	See page 345	Yellow powder, soluble in water	In micro-organisms as active group of flavoproteins, and in plants and animals. Makes up 5–30% of total riboflavin of animal organs ⁴
Flavinadenine dinucleotide (FAD)	See page 345	See page 345	Yellow powder, readily soluble in water, insoluble in ethanol	In micro-organisms as active group of flavoproteins, and in plants and animals. Makes up 70–90% of riboflavin of animal organs ⁴
Lumiflavin (7,8,10-trimethyl- isaloioxazine)	C ₁₉ H ₁₄ N ₄ O ₂ 256.27		Yellow crystals, only slightly soluble in water. Blue fluorescence in solution M. p. 333 °C (decomp.)	Formed from riboflavin by irradiation in alkaline solution. Antagonist of riboflavin
Galactoflavin (7,8-dimethyl- 10-[D-glucityl]- isaloioxazine)	C ₁₈ H ₁₈ N ₄ O ₇ 406.40		Yellow crystals, slightly soluble in water	Antagonist of riboflavin

per day, 10% of the intake is excreted; with an intake of 1.5 mg, about 20% is excreted, with an intake of 5–11 mg, about 60%^{17,19} (see also page 676). The concentration of riboflavin in breast milk depends on the intake of the vitamin²⁰.

Function

In the form of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) riboflavin forms the active group of the flavoproteins, enzymes with an important function in biological oxidations (see pages 402–403). Here the isoalloxazine system acts as a reversible redox system. In the oxidized form (fluorescent) the flavoproteins take up two hydrogen atoms and pass into the leucoform (non-fluorescent); in some reactions they take up only one hydrogen atom and become semiquinones. At least 40 flavoproteins

are known²¹, among them (active group in brackets) important oxidases such as aldehyde oxidase (FAD), xanthine oxidase (FAD), L-amino acid oxidase (FMN), D-amino acid oxidase (FAD), dehydrogenases like acyl-CoA-dehydrogenase (FAD) and succinate dehydrogenase (FAD), NAD(P)H₂ dehydrogenases (FAD), and glutathione reductase (FAD).

Riboflavin, in combination with protein, is necessary to prevent recurrent skin lesions such as those occurring in the corners of the mouth¹. In erythropoiesis, it is possibly necessary for the formation or effective functioning of erythropoietin²².

Requirements and deficiency symptoms

The requirement of riboflavin is usually based on the energy requirement²³ but can also be related to the protein requirement²⁴.

Depletion-repletion studies indicate that flavin are met by a daily intake of 0.44 mg figure that the daily intake of 0.55 mg, a Joint FAO/WHO Expert Group²⁴ i Food and Nutrition Board (USA)²⁴ i (page 494) on studies of riboflavin dependence of these on 'metabolic body size' power of the body weight: infants 0.009 mg, children 12–14 years 0.08 mg kg^{0.74}. For the requirements of pregnant the table on page 494.

Good sources of riboflavin are milk, yeast and green vegetables (see pages 49–50) stable to cooking, but up to 85% of the exposure to sunlight².

Experimental riboflavin deficiency is of the intestinal mucosa (glossitis, in pharyngeal mucosa, cheilitis, rhagades of the skin (severe pruritis, desquamation of the skin creases over joints, seborrheic dermatitis of the anogenital region (inflammation of the perianal region), vulva and scrotum also indicated injury to the bone marrow (leucocytes) with normochromic and normocytic anemia. Whether the vitamin deficiency (ariboflavinosis) are due to actual deficiency or to a nutritional defect. Biochemically, riboflavin deficiency is recognized by the low riboflavin content (276 µg/l at a daily intake of 2.55–3.55 mg intake of 0.55 mg²⁵) and by the reduced vitamin (below 40 µg/24 h²⁶).

Treatment

The deficiency symptoms usually disappear giving riboflavin in doses of 5 mg 2–3 times a day; this treatment will confirm the diagnosis.

Vitamin B₂^{1,2} (for references see page 47)

Chemistry

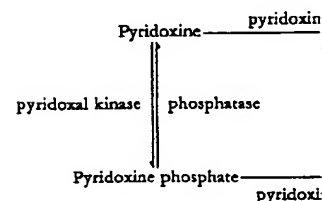
For structure and properties of vitamin B₂ see the table on page 474.

Assay^{3,4}

Biological. Growth test on chicks and deficiency dermatitis.

Microbiological. With *Saccharomyces cerevisiae* pyridoxine, pyridoxamine and pyridoxal 46%, pyridoxamine 40% of activity (casei (pyridoxal activity only), *Streptococcus* and pyridoxal activity only), *Tetrahymena* and pyridoxal activity only).

Enzymatic. Pyridoxal phosphate by nase.



Physical properties	Occurrence and activity
yellow bitter taste, soluble in ethanol, soluble in water, stable to heat and to acid, very unstable in light, green in urine	Constituent of flavin mononucleotide and flavin adenine dinucleotide. Occurs in free form in some micro-organisms. Makes up 0.5-2% of total riboflavin of animal organs ⁴ . Present in urine
5-282 °C	
powder, soluble in water	In micro-organisms as active group of flavoproteins, and in plants and animals. Makes up 5-30% of total riboflavin of animal organs ⁴
powder, soluble in water, insoluble in	In micro-organisms as active group of flavoproteins, and in plants and animals. Makes up 70-90% of riboflavin of animal organs ⁴
crystals, only soluble in blue fluorescent solution	Formed from riboflavin by irradiation in alkaline solution. Antagonist of riboflavin
crystals, soluble in	Antagonist of riboflavin

(active group in brackets) important dase (FAD), xanthine oxidase (FAD),), D-amino acid oxidase (FAD), dehydrogenase (FAD) and succinate (P)H₂ dehydrogenases (FAD), and

with protein, is necessary to prevent those occurring in the corners of the s possibly necessary for the formation rthropoietin²².

cy symptoms
avin is usually based on the energy
related to the protein requirement²⁴.

Depletion-repletion studies indicate that the body's needs of riboflavin are met by a daily intake of 0.44 mg/1000 kcal, and it is on this figure that the daily intake of 0.55 mg/1000 kcal recommended by a Joint FAO/WHO Expert Group²⁵ is based (see page 493). The Food and Nutrition Board (USA)²⁶ base their recommendations (page 494) on studies of riboflavin requirements that point to dependence of these on 'metabolic body size', represented as the 0.75th power of the body weight: infants 0.1 mg, children 10-12 years 0.09 mg, children 12-14 years 0.08 mg, adults 0.07 mg riboflavin/kg²⁷. For the requirements of pregnant and lactating women see the table on page 494.

Good sources of riboflavin are milk, liver, kidneys, heart, protein and green vegetables (see pages 499-515). The vitamin is fairly stable to cooking, but up to 85% of that in milk is destroyed on exposure to sunlight².

Experimental riboflavin deficiency in man has resulted in lesions of the intestinal mucosa (glossitis, inflammation of the buccopharyngeal mucosa, cheilitis, rhagades of the corners of the mouth), of the skin (severe pruritis, desquamation, rhagades, inflammation of the skin creases over joints, seborrheic dermatitis) and particularly of the anogenital region (inflammation, desquamation and severe pruritis of anus, vulva and scrotum); recent studies^{27, 28} have also indicated injury to the bone marrow (absence of mature erythrocytes) with normochromic and normocytic anaemia and deficiency of reticulocytes. Whether the vascularization of the cornea and capillary dilatation of the skin seen in endemic riboflavin deficiency (ariboflavinosis) are due to actual deficiency of the vitamin is uncertain^{1, 22}, and these symptoms may be the result of a multiple nutritional defect. Biochemically, riboflavin deficiency can be recognized by the low riboflavin content in the erythrocytes (202-276 µg/l at a daily intake of 2.55-3.55 mg, 100-131 µg/l at a daily intake of 0.55 mg²⁹) and by the reduced urinary excretion of the vitamin (below 40 µg/24 h³⁰).

Treatment

The deficiency symptoms usually disappear after several days of giving riboflavin in doses of 5 mg 2-3 times a day². The results of this treatment will confirm the diagnosis.

Vitamin B₂^{1, 2} (for references see page 476)

Chemistry

For structure and properties of vitamin B₂ and related compounds see the table on page 474.

Assay^{3, 4}

Biological. Growth test on chicks and rats, curatively on rats with deficiency dermatitis.

Microbiological. With *Saccharomyces carlsbergensis* (same activity for pyridoxine, pyridoxamine and pyridoxal), *S. cerevisiae* (pyridoxal 46%, pyridoxamine 40% of activity of pyridoxine), *Lactobacillus casei* (pyridoxal activity only), *Streptococcus faecalis* (pyridoxamine and pyridoxal activity only), *Tetrahymena pyriformis*⁵ (pyridoxamine and pyridoxal activity only).

Enzymatic. Pyridoxal phosphate by means of tyrosine decarboxylase.

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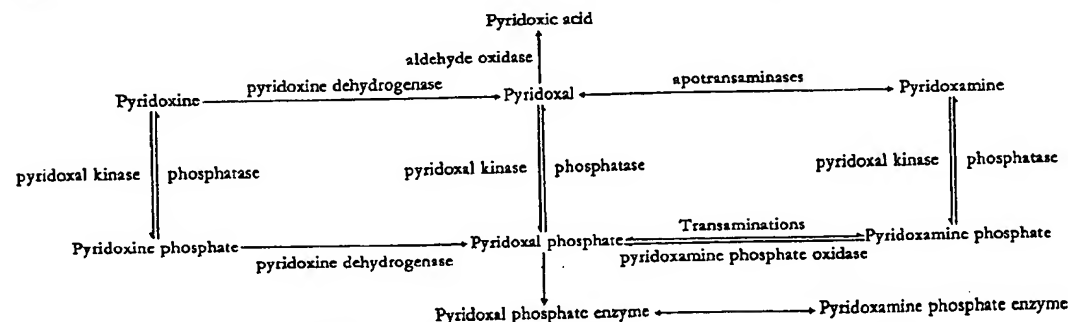
Chemical⁶. Spectrophotometrically or colorimetrically in pure solution on the basis of the reactivity of the phenolic hydroxyl group; pyridoxal and pyridoxal phosphate fluorimetrically as cyanohydrin (pyridoxine is converted into pyridoxal by oxidation, pyridoxamine to the same compound by transamination); pyridoxic acid fluorimetrically as lactone (pyridoxamine can be converted into pyridoxine; pyridoxine and pyridoxal can be oxidized to pyridoxic acid); all the above forms of the vitamin can be separated by column, paper or thin-layer chromatography.

Unit

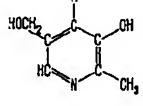
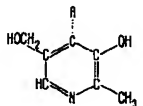
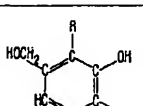
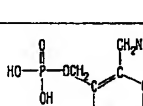
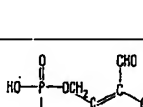
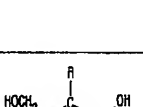
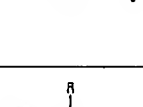
No international unit; by weight.

Biogenesis⁷

Practically nothing is known of the biosynthesis of the pyridine ring. Pyridoxine, pyridoxal, pyridoxamine and the phosphates are interconvertible in animal tissues and by micro-organisms in accordance with the scheme shown⁸.



Structure and properties of vitamin B₆ and related compounds

Names*	Formula and mol. wt.	Structure	Physical properties	Occurrence and activity
Pyridoxine*, pyridoxol* (adermine)	C ₈ H ₁₁ NO ₃ 169.18	 R = -CH ₂ OH	Colourless crystals, water-soluble, stable to heat, unstable to light M. p. 160 °C	Particularly in plants. Vitamin B ₆ activity for higher plants and yeasts; only slight activity for bacteria
Pyridoxamine*	C ₈ H ₁₁ N ₂ O ₃ 168.20	 R = -CH ₂ NH ₂	Colourless crystals, water-soluble, unstable to heat and light M. p. 193 °C	Particularly in animal tissues. Vitamin B ₆ activity for micro-organisms and higher animals
Pyridoxal*	C ₈ H ₉ NO ₃ 167.17	 R = -CHO	Colourless crystals, water-soluble, unstable to heat and light	Particularly in animal tissues. Vitamin B ₆ activity for micro-organisms and higher animals
Pyridoxamine phosphate	C ₈ H ₁₂ N ₂ O ₅ P 248.18		—	Coenzyme in transaminations
Pyridoxal phosphate (codecarboxylase)	C ₈ H ₁₀ NO ₅ P 247.15		Yellow crystals, water-soluble M. p. > 270 °C	Particularly in muscle. Coenzyme in decarboxylations, transaminations and phosphorylations
Pyridoxic acid (4-pyridoxic acid)	C ₈ H ₉ NO ₄ 183.17	 R = -COOH	White crystals, moderately soluble in water M. p. 247 °C	Particularly in urine (breakdown product). No vitamin B ₆ activity
Deoxypyridoxine	C ₈ H ₁₁ NO ₃ 153.18	 R = -CH ₃	—	Vitamin B ₆ antagonist in micro-organisms and animals

* Trivial names recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry [*Biochim. biophys. Acta (Amst.)*, 107, 1 (1965)].

Intake and excretion

An average daily diet contains about 1 mg B₆ phosphates from foods are probably absorbed by phosphatases¹, and the non-phosphorylated B₆ is absorbed in the upper intestinal tract also by the intestinal flora, though it makes use of this source of the vitamin in the faeces is largely independent of adults to 0.7–0.9 mg per day and in children 2. For urinary excretion of the vitamin see page 639.

The non-phosphorylated compounds are found in the tissues (mainly the brain, liver, kidney, heart, muscle, and blood), but not in the blood, by the action of phosphatases and ATP^{1,2}. The vitamin B₆ activity in the serum (ca. 10 µg/l) and leucocytes (ca. 20–45 µg/l) of the serum 30–80 µg/l^{1,2}. P. The vitamin B₆ does not seem to be present in the plasma results within 3 days in an inpatient content of whole blood and within the leucocytes². The human body contains 7 mg pyridoxine per day into the body, and intakes of pyridoxine do not cause any change in the phosphate content of the blood¹.

Vitamin B₆ is stored in the liver (5–20 µg/g) and brain (12–25 µg/g)^{1,2}, and it may be excreted in the urine (see page 639). The vitamin B₆ content of the body is estimated variously at 40–150 mg^{1,4} and the pyridoxal phosphate in the body at 100–150 mg^{1,4}. The vitamin B₆ is 2.2–4.4%, with a 2–3% depletion of the body stores.

Pyridoxal is converted in the liver to pyridoxic acid, which is excreted in the urine of pyridoxine, pyridoxal and pyridoxamine. The normal diet about a half or less of the vitamin B₆ is excreted in the urine^{1,4,17}.

Function^{1,18}

Vitamin B₆ is involved as coenzyme in many reactions^{1,18}. Pyridoxamine phosphate and pyridoxal phosphate are coenzymes in transamination reactions of amino acids and for other reactions (see page 639). The vitamin B₆ content of the body is estimated variously at 40–150 mg^{1,4} and the pyridoxal phosphate in the body at 100–150 mg^{1,4}. The vitamin B₆ is 2.2–4.4%, with a 2–3% depletion of the body stores.

Pyridoxal phosphate is a component of α-glucanase and other enzymes. Deoxypyridoxine and other synthetases (such as isonicotinic acid hydrazide) are B₆ antagonists¹⁸, with various degrees of antagonism linked to pyridoxal phosphate.

Enzymatic reactions with pyridoxamine phosphate

Enzyme	Reaction
Diaminooxidase, histaminase	Oxidative
Serine hydroxymethyl transferase	Formic acid, hydrofolate
α-Glucan phosphorylase	Phosphogluconate
Transaminases	Amino acids, amino acids, and amino acids
Synthases	Formic acid and indole, methionine, and methionine

* Pyridoxamine phosphate acts as cofactor

Physical properties	Occurrence and activity
Less crystals, soluble, stable, unstable to 60 °C	Particularly in plants. Vitamin B ₆ activity for higher plants and yeasts; only slight activity for bacteria
Less crystals, soluble, stable to heat and 93 °C	Particularly in animal tissues. Vitamin B ₆ activity for micro-organisms and higher animals
Less crystals, soluble, stable to heat and	Particularly in animal tissues. Vitamin B ₆ activity for micro-organisms and higher animals
	Coenzyme in transaminations
Less crystals, soluble, > 270 °C	Particularly in muscle. Coenzyme in decarboxylations, transaminations and phosphorylations
Crystals, slightly soluble at 247 °C	Particularly in urine (breakdown product). No vitamin B ₆ activity
	Vitamin B ₆ antagonist in micro-organisms and animals

From Pure and Applied Chemistry and the Inter-

Intake and excretion

An average daily diet contains about 2 mg vitamin B₆². Vitamin B₆ phosphates from foods are probably hydrolysed in the intestine by phosphatases³, and the non-phosphorylated compounds undergo absorption in the upper intestinal tract. Vitamin B₆ is formed also by the intestinal flora, though it is hardly likely that the body makes use of this source of the vitamin¹⁰. Excretion of vitamin B₆ in the faeces is largely independent of the intake and amounts in adults to 0.7–0.9 mg per day and in children to 0.15–0.30 mg per day². For urinary excretion of the vitamin see page 676.

The non-phosphorylated compounds are converted into the phosphates in the tissues (mainly the brain, liver and kidneys), but probably not in the blood, by the action of the enzyme pyridoxal kinase and ATP¹¹. The vitamin B₆ activity of whole blood amounts to 20–45 µg/l, of the serum 30–80 µg/l¹². Pyridoxal phosphate occurs in the serum (ca. 10 µg/l) and leucocytes (see also pages 609–610). Pyridoxine does not seem to be present in the blood⁴. Pyridoxine administration results within 3 days in an increase in the pyridoxal phosphate content of whole blood and within 10 days in a similar increase in the leucocytes². The human body can apparently convert a maximum of 7 mg pyridoxine per day into pyridoxal phosphate; higher intakes of pyridoxine do not cause any further increase in the pyridoxal phosphate content of the blood¹³.

Vitamin B₆ is stored in the liver (5–20 µg/g), muscles (2–6 µg/g) and brain (12–25 µg/g)¹², and it may be present in the spinal fluid (see page 639). The vitamin B₆ content of the whole body has been estimated variously at 40–150 mg¹⁴ and 16–32 mg¹⁵; about a half of the pyridoxal phosphate in the body appears to be bound to α-glucan phosphorylase in the muscles¹⁶. The daily turnover of vitamin B₆ is 2.2–4.4%, with a 2–3% depletion of the body's reserves¹⁴.

Pyridoxal is converted in the liver by aldehyde oxidase to pyridoxic acid, which is excreted in the urine along with small amounts of pyridoxine, pyridoxal and pyridoxamine (see page 676). On a normal diet about a half or less of the vitamin B₆ turnover appears as pyridoxic acid in the urine^{14,17}.

Function^{6,18}

Vitamin B₆ is involved as coenzyme in over 40 enzymatic reactions¹⁹. Pyridoxamine phosphate and pyridoxal phosphate act as coenzymes in transamination reactions important for the breakdown of γ-aminobutyric acid in the brain and for oxalic acid metabolism. Pyridoxal phosphate is the coenzyme in the decarboxylation of amino acids and for other reactions of amino acids (see the table below). Pyridoxal phosphate is also involved in various reactions of tryptophan metabolism (see figure), and this fact is made use of in the tryptophan loading test for diagnosis of vitamin B₆ deficiency²⁰. Pyridoxal phosphate is also the coenzyme in the transport of one-carbon units from serine to tetrahydrofolic acid²¹, and plays a role in the formation of circulating antibodies²². It is also involved with other cofactors in the synthesis of 8-aminolaevulinic acid, a precursor of the porphyrins (haemopoiesis)²³. Pyridoxal phosphate is a component of α-glucan phosphorylase¹⁶. It is uncertain whether vitamin B₆ is directly involved in fat metabolism²⁴.

Deoxypyridoxine and other synthetic pyridoxines, hydrazides (such as isonicotinic acid hydrazide) and cycloserine act as vitamin B₆ antagonists²⁵, with various degrees of inactivation of the enzymes linked to pyridoxal phosphate.

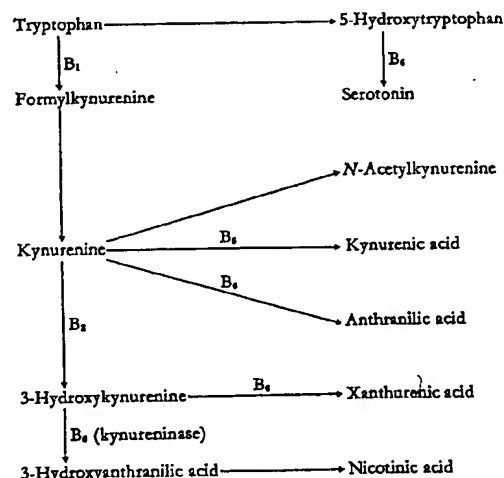
Enzymatic reactions with pyridoxamine phosphate and pyridoxal phosphate as cofactors*

Enzyme	Reaction	Enzyme	Reaction
Diaminoxidase, histaminase	Oxidation of diamines and histamine	Decarboxylases	For example, decarboxylation of histidine to histamine, tyrosine to tyramine, dopa to dopamine, hydroxytryptophan to serotonin
Serine hydroxymethyl transferase	Formation of 5,10-methylene tetrahydrofolic acid	Threonine aldolase	Breakdown of threonine to glycine and acetaldehyde
α-Glucan phosphorylase	Phosphorolysis of glycogen	Dehydratases	Deamination of serine, homoserine, threonine, etc.
Transaminases	amino acid + ketoic acid ⇌ ketoic acid + amino acid (with all naturally occurring amino acids)	Desulphydrases	Deaminating desulphydration of cysteine and homocysteine
Synthases	Formation of tryptophan from serine and indole, of cysteine from serine, of methylcysteine from serine and methanethiol	Racemases	L-amino acid ⇌ D-amino acid (alanine, methionine, glutamic acid)

* Pyridoxamine phosphate acts as cofactor only in transaminations.

Dependence of tryptophan metabolism on the B vitamins^{20, 26}

Bold arrows indicate the main breakdown route. In vitamin B₆ deficiency, kynureninase is inactivated more strongly than the transaminases, which are involved in the formation of xanthurenic acid²⁷.



Requirements and deficiency symptoms

In adults the minimum requirement of pyridoxine hydrochloride is apparently about 1.25 mg per day with a daily protein intake of 30 g and 1.5 mg per day with a daily protein intake of 100 g, the optimal requirement being 1.75–2.0 mg per day with a daily protein intake of 100 g^{28, 29}. The requirement of the vitamin increases with the protein intake. In infants it is between 0.1 and 0.5 mg per day and is dependent on the protein intake (20 µg/g protein); the minimum requirement of children is 0.5–1.5 mg per day, that of adolescents 1.5–2 mg per day³⁰. The Food and Nutrition Board (USA) recommend a vitamin B₆ intake of 2.0 mg per day when the daily protein intake is 100 g or more³⁰. The human vitamin B₆ requirement has been the object of much discussion^{31, 32}; it is possible that even higher intakes than those given above are desirable. During pregnancy the requirement is probably increased³³, and a daily intake of 2.5 mg has been recommended for pregnant and lactating women³⁰. See also page 494.

Vitamin B₆ occurs in practically all vegetable and animal food-stuffs (see pages 499–515). Good sources of vitamin B₆ are yeast, liver and cereals (wholemeal, since milling results in loss of 80–90% of the vitamin)².

The symptoms of vitamin B₆ deficiency vary greatly with the species and age of the individual. The great variety of deficiency symptoms observed is in part due to the fact that in progressive B₆ deficiency not all the enzyme systems are blocked simultaneously to

the same extent²⁷. The following symptoms have been observed in experimental B₃ deficiency²⁷: in rats, severe dermatitis (rat pellagra), occasionally haemolytic anaemia and overall loss of body fat; in rabbits, desquamating dermatitis of the ears, mild anaemia, convulsions, creatinuria, paralytic collapse and death²⁸; in rhesus monkeys, arteriosclerosis, dental caries, fatty degeneration or cirrhosis of the liver, pancreatic sclerosis, disturbances of the central nervous system²⁹; in man, (a) skin and mucosa: seborrhoeic and desquamative dermatitis of the mouth and eyes which may spread to the face, scalp, neck and loins; intertrigo of the breasts and inguinal region in women; stomatitis and glossitis; (b) nervous system: irritability, depression, somnolence, nausea, impairment of sensitivity to vibration and positional change; very rarely peripheral neuritis³⁷.

Spontaneous vitamin B₃ deficiency is rare in man. Among 300 infants who received only about 60 µg vitamin B₃ per litre of formula milk (as a result of a new sterilization process) hyperacusis, nervousness and epileptiform convulsions were observed³⁰. Of possible genetic origin are the pyridoxine-dependent convulsions seen in infants (generalized convulsions with no clinical or electroencephalographic peculiarities appearing in the first days of life and occasionally developing into status epilepticus during the following weeks)³⁰⁻³²; these are probably due to an unsatisfied vitamin B₃ requirement or to a disturbance of vitamin B₃ utilization. Similar causes are probably at the root of the pyridoxine-deficiency anaemia and the pyridoxine-sensitive anaemia seen in man^{33, 41, 42}, but in contrast to the central nervous disturbances these are observed almost solely among adults. In pyridoxine-deficiency anaemia (a hypochromic microcytic anaemia with increased serum iron and organ haemosiderosis) there is a disturbance of δ-aminolaevulinic acid synthesis, with consequent reduction in the amount of protoporphyrin formed; this disease is also probably of genetic origin. In pyridoxine-sensitive anaemia (symptoms as in the deficiency anaemia but often including enlargement of the liver and spleen) there is a complex disturbance of porphyrin metabolism resembling that in sideroblastic anaemia.

It is uncertain whether vitamin B₃ deficiency in man causes dental caries⁴³ or the formation of oxalate stones in the urinary tract⁴⁴.

Cystathionuria is probably due to a defect in the linkage of the coenzyme pyridoxal phosphate to the apoenzyme of homoserine dehydratase (cystathionase)⁴⁵.

Biochemically, vitamin B₃ deficiency is recognizable (a) by the increased excretion of xanthurenic acid and other tryptophan metabolites in urine, especially following oral doses of tryptophan (tryptophan loading test)²⁰; this disturbance of tryptophan metabolism appears after only one week on a vitamin B₃-deficient diet⁴⁶; (b) by a lowered pyridoxal phosphate level in the blood and a greatly reduced vitamin B₃ and pyridoxic acid excretion in the urine⁴⁷; (c) by a reduced transaminase content of the serum⁴⁷ and erythrocytes⁴⁸; (d) by an increased urinary oxalic acid excretion^{44, 49} and a lowered urinary taurine excretion⁴⁹.

Treatment

When the vitamin B₃ deficiency is purely alimentary, daily doses of the vitamin at the level of the normal requirement suffice. In pyridoxine-dependent convulsions in infants pyridoxine should be given parenterally at the rate of 2-15 mg per day⁴⁰; in pyridoxine-deficiency anaemia the dose should be at least 10 mg per day⁴¹ and in pyridoxine-sensitive anaemia at least 500 mg per day⁴¹. Daily pyridoxine supplements of 10-15 mg may be helpful in overcoming disturbances of pregnancy such as severe vomiting and toxemia, particularly when the diet is poor⁵⁰. Pyridoxine doses of 100 mg have been recommended for the treatment of radiation sickness⁵¹. The success of treatment with pyridoxine is conditional on there being no disturbance of the conversion into pyridoxal (by the action of pyridoxine dehydrogenase) in the body⁶.

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Nicotinic acid¹⁻³ (for references see page 478)

Chemistry

For structure and properties of nicotinic acid and related compounds see the table opposite.

Assay

Biological⁴. Black tongue curative test in dogs, growth test in chicks.

Microbiological⁵. With *Lactobacillus plantarum* (earlier *L. arabinosus*) or *Tetrahymena pyriformis* for nicotinic acid and nicotinamide.

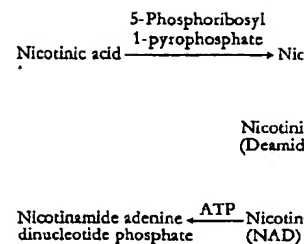
Chemical⁶. In pure solution spectrophotometrically, polarographically, by mass analysis, colorimetrically by the bromcyanogen (KÖNIG) reaction or fluorometrically (the latter method particularly suitable for organs). Nicotinic acid and its metabolites can be separated by chromatographic methods. NAD and NADP must be hydrolysed before being determined as nicotinic acid; they can be estimated directly by the spectrophotometric or fluorimetric method through their blue fluorescence in alkaline solution.

Unit

No international unit; by weight.

Biogenesis^{7, 8}

In plants nicotinic acid arises by condensation of 3- and 4-carbon units. In animals, fungi and a few bacteria (for instance, *Xanthomonas pruni*) nicotinic acid is formed from tryptophan by the action of thiamine, riboflavin and vitamin B₆ (see diagram on page 475). It is less likely that nicotinamide is formed directly from nicotinic acid than by the hydrolysis of nicotinamide dinucleotides. The latter compounds are formed in erythrocytes, liver, yeast, etc. in accordance with the following scheme:



Intake and excretion

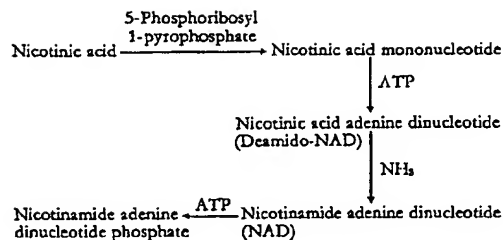
In the USA the daily average diet contains 8-17 mg nicotinic acid⁹. Nicotinic acid is readily absorbed in the intestine, though it is unlikely that the human body can synthesize it. In the body about 1 mg nicotinic acid is excreted¹⁰; the enzymes required for this are in the liver and erythrocytes. This conversion of nicotinic acid to nicotinamide is increased in pregnancy¹¹.

The nicotinic acid content of whole body is about 100 mg. The serum contains about 0.02-0.05 mg/ml (also page 610). The nicotinic acid level in urine is about 0.02-0.05 mg/ml when nicotinic acid is given parenterally.

Structure and properties of nicotinic acid and

Compound	Formula mol. wt.
Nicotinic acid* (nicotinic acid, pyridine-3-carboxylic acid, vitamin PP)	C ₆ H ₅ N ₂ O ₂ 123.11
Nicotinamide* (nicotinamide, nicotinamide, pyridine-3-carboxylamide, vitamin PP)	C ₆ H ₇ N ₃ O ₂ 122.13
1-Methylnicotinamide (N ₁ -methylnicotinamide)	C ₇ H ₈ N ₂ O ₂ 137.16
1-Methyl-6-pyridone-3-carboxylamide (N ₁ -methyl-2-pyridone-5-carboxylamide)	C ₇ H ₈ N ₂ O ₂ 152.15
Nicotinamide adenine dinucleotide (NAD; reduced form NADH ₂)	See page.
Nicotinamide adenine dinucleotide phosphate (NADP; reduced form NADPH ₂)	See page.

* Trivial names recommended by the Commission of Biochemistry [Biochim. biophys. Acta]



Intake and excretion

In the USA the daily average diet contains about 500–1000 mg tryptophan and 8–17 mg nicotinic acid⁸. Nicotinic acid and tryptophan are readily absorbed in the intestinal tract. Nicotinic acid is probably synthesized from tryptophan by the intestinal bacteria, though it is unlikely that the human body makes use of this source. In the body about 1 mg nicotinic acid is formed from every 60 mg tryptophan^{11,12}; the enzymes required for this transformation occur in the liver and erythrocytes. This conversion of tryptophan into nicotinic acid is increased in pregnancy¹⁰.

The nicotinic acid content of whole blood is about 4–10 mg per litre and consists almost wholly of dinucleotides in the blood cells. The serum contains about 0.02–0.05 mg of free nicotinic acid⁸ (see also page 610). The nicotinic acid level in the blood increases rapidly when nicotinic acid is given parenterally; oral doses of nicotinic

acid or tryptophan raise the dinucleotide content of the erythrocytes. In the form of dinucleotides, nicotinic acid occurs in all tissues, particularly the liver. The human liver contains on the average 65 mg nicotinic acid¹¹.

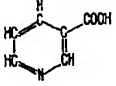
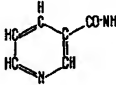
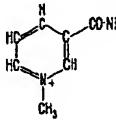
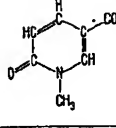
In the liver, nicotinamide but not nicotinic acid is methylated to 1-methylnicotinamide with S-adenosylmethionine as methyl-group donor and is further oxidized to 1-methyl-2-pyridonecarboxylamide⁸. These compounds are excreted in the urine (per day normally 5–8 mg 1-methylnicotinamide and 7–10 mg 1-methyl-2-pyridonecarboxylamide as well as about 1 mg nicotinic acid²; see also page 676). After oral doses of 10–150 mg nicotinamide per day, on the average 57% has been found in the urine, consisting of 10–30% 1-methylnicotinamide and 70–90% 1-methyl-2-pyridonecarboxylamide¹². Following high doses of nicotinic acid but not nicotinamide, nicotinic acid is also excreted in the urine bound to glycine (as nicotinic acid).

Function

The active forms of nicotinic acid are the nicotinamide dinucleotides NAD and NADP. These are coenzymes (cosubstrates) of numerous dehydrogenases, particularly in fermentation, glycolysis and other reactions. They are responsible also for hydrogen transport within the cell, NADPH₂ providing the hydrogen necessary for biosynthesis while NADH₂ transports this hydrogen to the enzymes of the respiratory chain. The synthesis of ATP utilizes the reaction with oxygen to form water (oxidative phosphorylation); for details see pages 403–404.

Nicotinic acid but not nicotinamide has an inhibiting effect at high dosage on the synthesis of lipids, particularly cholesterol, but

Structure and properties of nicotinic acid and related compounds

Compound	Formula and mol. wt.	Structure	Physical properties	Occurrence and activity
Nicotinic acid* (niacin, pyridine-3-carboxylic acid, vitamin PP)	C ₆ H ₅ NO ₂ 123.11		White crystals, acid taste, moderately soluble in water and ethanol, stable to heat and oxidation M.p. 234–237 °C	In plant and animal tissues; component of NAD and NADP
Nicotinamide* (nicotinic acid amide, niacinamide, pyridine-3-carboxylamide, vitamin PP)	C ₆ H ₆ N ₂ O 122.13		White crystals, salty taste, soluble in water and ethanol, stable to heat and oxidation M.p. 128–131 °C	In plant and animal tissues; component of NAD and NADP
1-Methylnicotinamide (N ₁ -methylnicotinamide)	C ₇ H ₈ N ₂ O 137.16			In urine; metabolite of nicotinic acid
1-Methyl-6-pyridone-3-carboxylamide (N ₁ -methyl-2-pyridone-5-carboxylamide)	C ₇ H ₈ N ₂ O ₂ 152.15		White crystals, soluble in water and ethanol M.p. 212–214 °C	In urine; metabolite of nicotinic acid
Nicotinamide adenine dinucleotide (NAD; reduced form NADH ₂)	See page 344	See page 344	Colourless powder, soluble in water, insoluble in ethanol	In all animal and plant cells; coenzyme of many dehydrogenases
Nicotinamide adenine dinucleotide phosphate (NADP; reduced form NADPH ₂)	See page 344	See page 344	Colourless powder, soluble in water, insoluble in ethanol	In all animal and plant cells; coenzyme of many dehydrogenases

* Trivial names recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry [*Biochim. biophys. Acta (Amst.)*, 107, 1 (1965)].

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ies of nicotinic acid and related com-
ic.

curative test in dogs, growth test in

obacillus plantarum (earlier L. arabinosus)
nicotinic acid and nicotinamide.

spectrophotometrically, polarograph-
irometrically by the bromcyanogen
etrically (the latter method particularly
ic acid and its metabolites can be sep-
methods. NAD and NADP must be
ermined as nicotinic acid; they can be
trophotometric or fluorimetric method
nce in alkaline solution.

weight.

ses by condensation of 3- and 4-carbon
a few bacteria (for instance, Xantho-
formed from tryptophan by the action
itamin B₆ (see diagram on page 475).
nide is formed directly from nicotinic
nicotinamide dinucleotides. The latter
erythrocytes, liver, yeast, etc. in accor-
sime:

the mechanism of this effect is obscure¹²; the primary action of nicotinic acid is possibly the liberation of free fatty acids blocked in the tissues¹⁴.

Requirements and deficiency symptoms

The nicotinic acid requirement can also be met by tryptophan, 60 mg of which corresponds to 1 mg nicotinic acid. The requirement of nicotinic acid depends on the caloric intake. The minimum requirement for preventing pellagra is 4.4 mg per 1000 kcal, or 9 mg for adults whose daily caloric intake is below 2000 kcal². For children and adults the Food and Nutrition Board (USA)² recommend a daily intake of 6.6 mg per 1000 kcal; during pregnancy this should be increased by 2 mg per day, during lactation by 7 mg per day (see page 494). The same daily intake of 6.6 mg/1000 kcal for children and adults is recommended by the Joint FAO/WHO Expert Group²² (see page 493); for infants the latter accept that breast feeding by well-nourished mothers will supply adequate niacin equivalents.

Good sources of nicotinic acid are yeast, liver, lean meat, groundnuts and leguminous plants (see pages 499-515). Plant proteins contain 0.8-1.4% tryptophan, animal proteins about 1.3% (see page 516). Maize is low in both nicotinic acid and tryptophan; nicotinic acid is also present in the combined form so that it is not available to the organism¹⁸. During the roasting of coffee considerable amounts of nicotinic acid are formed from trigonelline¹⁹.

Nicotinic acid deficiency causes pellagra, the development of which is favoured by sunlight and heavy physical work. Alimentary nicotinic acid deficiency is common in areas where maize constitutes the principal foodstuff. Pellagra occasionally occurs in chronic alcoholism, cirrhosis of the liver, chronic diarrhoea, diabetes and neoplasias. In the presence of carcinoid tumours up to 60% (normally 1%) of the body's tryptophan is converted into serotonin, so that it is no longer available as a source of nicotinic acid¹⁷. Treatment with isoniazid can cause inhibition of the activity of pyridoxal phosphate and thus interfere with the synthesis of nicotinic acid from tryptophan. This synthesis is possibly also impaired by diets containing large amounts of leucine¹⁸.

The following are the symptoms of pellagra: (a) A dark red erythema appearing symmetrically on the extremities, face, neck and all other regions exposed to air and light; the skin finally becomes dry, fissured, atrophic and brown-coloured. The lesions are marked by atrophy of the superficial layers of the corium with dilatation of the blood vessels, keratinization of the epidermis and a tendency for the latter to separate from the corium. Wounds of any kind exacerbate these symptoms. (b) Chronic inflammation of the mucosa and intestinal tract (stomatitis, glossitis, gastritis with low acid secretion); profuse and often bloody diarrhoea. (c) Emotional disturbances (delirium, hallucinations, confused mental states). Neurological disturbances, if present, are probably due to simultaneous deficiency of other vitamins since these symptoms have not been observed in experimental nicotinic acid deficiency².

The biochemical signs of nicotinic acid deficiency are the following: in pellagra a urinary excretion of 1-methylnicotinamide plus 1-methyl-2-pyridonecarboxylamide of usually less than 2 mg per day. Within 30-60 days the excretion of these metabolites falls to a minimum value and then remains constant; shortly after this minimum value is reached, the first clinical signs of deficiency appear². On a standard diet (10 mg nicotinic acid plus 1000 mg tryptophan) the excretion of nicotinic acid metabolites is less than 3.0 mg in pellagra patients and 7-37 mg in healthy persons¹⁹. In nicotinic acid deficiency the concentration of nicotinamide dinucleotides in the muscles and liver falls, but not that in the erythrocytes^{2, 20}.

Treatment

In severe nicotinic acid deficiency, 300-500 mg nicotinamide should be given in daily oral doses of 50-100 mg; if there is difficulty in swallowing, 100 mg nicotinamide should be given three times per day intramuscularly². Nicotinic acid should not be given intravenously in doses exceeding 25 mg owing to the danger of anaphylactic shock. In high doses nicotinic acid but not nicotinamide causes marked dilatation of the vessels and particularly of the capillaries and vessels of the upper half of the body: this is of therapeutic use in disturbances of the peripheral circulation. Nicotinic acid can be used to lower the serum cholesterol level and for this purpose is usually given at the rate of 1 g three times per day²¹.

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Folic acid group¹⁻³

Chemistry⁴

For structure and properties of folic acid and related compounds see the table on pages 480-481.

Assay

Biological. Curative test on chickens⁵.

Microbiological^{1, 6}. With *Lactobacillus casei* (total folic acid activity: pteroylglutamic acid, pteroyltriglutamic acid and higher conjugates, reduced folic acid including 5-methyltetrahydropteroylglutamic acid); with *Streptococcus faecalis* (pteroylglutamic acid, reduced folic acid, but not 5-methyltetrahydropteroylglutamic acid); with *Pedococcus carnis* (reduced folic acid). In biological material, particularly foodstuffs, the conjugates can also be broken down enzymatically.

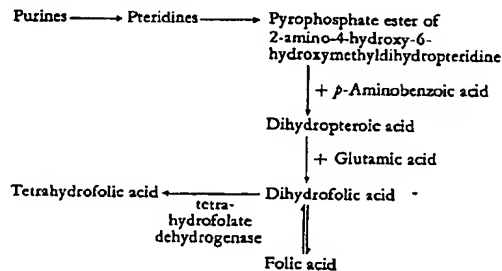
Chemical⁷. Photometrically after fission with zinc or potassium permanganate and subsequent diazotization; in pure solution also spectrophotometrically or polarographically.

Units

No international unit; by weight.

Biogenesis⁸

Folic acid is synthesized by higher plants, by micro-organisms (intestinal flora) and in animal tissues⁹, probably in accordance with the following scheme:



The biosynthesis of dihydrofolic acid is inhibited by sulphonamides¹⁰. Folic acid antagonists like aminopterin and substances with a structure resembling pyrimidine (e.g., primidone and pyrimethamine) inhibit tetrahydrofolate dehydrogenase and thereby the formation of tetrahydrofolic acid.

Intake and excretion

In USA the daily diet contains about 150-200 µg folic acid activity^{11, 12}; this includes only about 20 µg pteroylglutamic acid¹².

The folic acid conjugates are hydrolytically tract. Both the folic acid derived from the intestinal flora are actively absorbed; large amounts also diffuse passively¹³.

On the folic acid content of blood, see page 610. The maximum folic acid is attained 2-4 hours after an oral dose; life of intravenous doses in the serum is: administration of folic acid has no effect level; the exogenous folic acid is reduced in small amounts. The total folic acid has been estimated at 12-15 mg¹⁴; of this contained in the liver¹⁵. In serum^{16, 18}, folic acid is predominantly present in hydropteroylglutamic acid. The body's requirement to prevent the appearance of clinical 4-5 months¹⁹. After an intravenous dose active folic acid and 10-formyltetrahydrofolic acid have been found in the urine as well as unhydropteroylglutamic acid originating from

Function²³

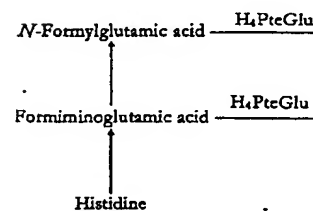
Tetrahydrofolic acid (H₄PteGlu) is a carbon units. These units arise mainly from the synthesis of purine and pyrimidine (see pages 433 and 436).

On account of the importance of folic acid synthesis it is understandable that essential part in all processes of cell division (stimulation of the reticulocytes) primary defect is probably a disturbance in the nucleus during cell division. The normal pregnancy is still observed alters the action of the ovarian hormone

Requirements

The folic acid requirement is not known. The minimum requirement of adults is probably sufficient to maintain the serum level²¹ requirement of about 5-20 µg²² or more increased in pregnancy (possibly 200-400 µg). In a deficiency of folic acid (Folic Acid Board (USA)²⁴ recommend a daily 0.8 mg for pregnant women, and 0.5 mg are amounts determined in foods by (see page 494).

Formate



Treatment

Treatment of folic acid deficiency should be adjusted as closely as possible to the deficiency²⁵. In megaloblastic anaemia due to vitamin B₁₂ deficiency, 0.25 mg folic acid per day is blood picture²⁶. Doses of folic acid are required to prevent the appearance of vitamin B₁₂ deficiency but are inadequate system from subacute degenerative disease employing folic acid antagonists (car given in reduced form (for example pregnancy, prophylactic doses of 0.1-1 mg)²⁷.

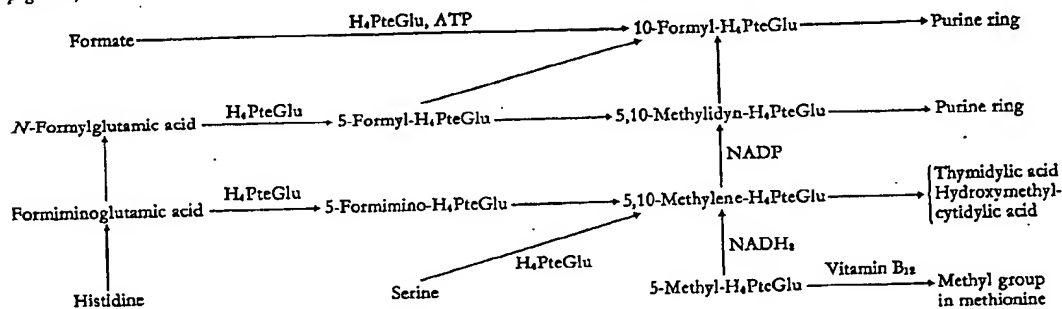
contains about 150–200 μg folic acid
only about 20 μg pteroylglutamic acid^{1,2}.

The folate acid requirement is not known for certain²⁶. The daily minimum requirement of adults is probably about 50 µg, an amount sufficient to maintain the serum level²⁵. Infants have a minimum requirement of about 5–20 µg²⁶ or more²⁷. The requirement is increased in pregnancy (possibly 200–400 µg per day in the 3rd trimester when there is a deficiency of folate)²⁸. The Food and Nutrition Board (USA)²⁴ recommend a daily intake of 0.4 mg for adults, 0.8 mg for pregnant women, and 0.5 mg for lactating women; these are amounts determined in foods by *Lactobacillus casei* assay (see page 494).

Deficiency symptoms appear in the following order³³:

Lowered serum folic acid level ($<7-16 \mu\text{g/l}$)	2 weeks
Excessive segmentation of the leucocytes	6-10 weeks
Increased excretion of formiminoglutamic acid	12-18 weeks
Lowered erythrocyte folic acid content	17 weeks
Macroelliptocytosis	18 weeks
Megaloblastosis of the bone marrow	19 weeks
Macrocytic anaemia	20 weeks

Secondary folic acid deficiency in vitamin B₁₂ deficiency is probably due to blocking of tetrahydrofolic acid regeneration from 5-methyltetrahydrofolic acid, a step requiring vitamin B₁₂.¹²



Treatment Treatment of folic acid deficiency with doses of the vitamin should be adjusted as closely as possible to the severity of the deficiency³⁴. In megaloblastic anaemia due to dietary deficiency of the vitamin, 0.25 mg folic acid per day is sufficient to normalize the blood picture³⁵. Doses of folic acid exceeding 0.1 mg per day are required to prevent the appearance of anaemia in patients with vitamin B12 deficiency but are inadequate to protect the nervous system from subacute degenerative changes^{34, 36}. In treatment employing folic acid antagonists (cancer) folic acid should be given in reduced form (for example as folinic acid)³⁷. During pregnancy, prophylactic doses of 0.1–0.5 mg folic acid are recommended¹⁶.


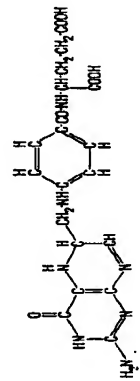
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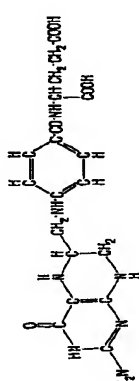
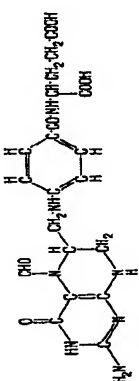
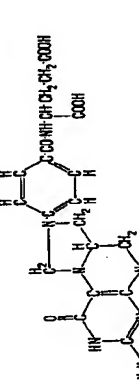
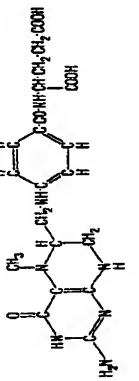
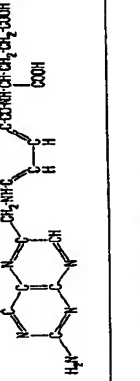
(continued on page 482)

Structure and properties of folic acid and related compounds

Names*	Formula and mol. wt.	Structure	Physical properties	Occurrence and biological properties
Rhizopterin (SLR factor)	$C_{13}H_{13}N_5O_4$ 340.30		Light-yellow platelets	In fermentation juice of <i>Rhizopus nigra</i> . Weak folic acid activity
Pteroylglutamic acid (folic acid, folacin, vitamin B ₉ , p-(2-amino-4-oxo-6-hydroxy-2,4-dihydropteridin-6-yl)-L-glutamic acid)	$C_{19}H_{19}N_5O_7$ 441.41		Orange-yellow needles or platelets, odourless and tasteless [α] _D ²⁰ +20° in 0.1-N NaOH	In liver, yeast, green leaves. Growth factor for <i>Lactobacillus casei</i> , <i>Streptococcus faecalis</i> K and other micro-organisms. Anti-anæmic properties
Pteroyltriglutamic acid, PteGlu ₃ (teropterin)	$C_{25}H_{25}N_5O_{12}$ 699.64		Light-yellow amorphous powder	In micro-organisms; formed in fermentations induced by corynebacteria. Weak folic acid activity
Pteroylheptaglutamic acid, PteGlu ₇ (vitamin B ₉ conjugate)	$C_{37}H_{37}N_5O_{18}$ 1216.11		Orange crystals	In yeast. Microbiologically inactive; presumably the form in which pteroylglutamic acid is stored
Dihydropteroylglutamic acid, H ₂ PteGlu (dihydrofolic acid, DHF)	$C_{19}H_{21}N_5O_7$ 443.42		Light-yellow amorphous powder	Intermediary metabolite

Tetrahydropteroylglutamic acid, H ₄ PteGlu (tetrahydrofolic acid, THF)	$C_{19}H_{23}N_5O_7$ 445.44		Pale cream-coloured powder, oxidizing in air, unstable to light, particularly in solution (-)-L-Form: [α] _D ¹⁵ -16.9°	Active form of folic acid
5-Formyltetrahydropteroylglutamic acid (citrovorum factor, folic acid, leucovorin)	$C_{20}H_{23}N_5O_7$ 473.45		Colourless crystals [α] _D ¹⁵ -15.1° (natural factor) [α] _D ¹⁵ +16.76° (racemate, ...)	In micro-organisms. Growth factor for <i>Leuconostoc citrovorum</i> , <i>Lactobacillus casei</i> , <i>Streptococcus faecalis</i> , <i>Lactobacillus arabinosus</i> ; carrier of ...

	$n = 6$		
Dihydropteroylglutamic acid, $H_2PteGlu$ (dihydrofolic acid, DHF)	$C_{20}H_{28}N_4O_6$ 443.42		Light-yellow amorphous powder
			Intermediate metabolite

Tetrahydropteroylglutamic acid, $H_4PteGlu$ (tetrahydrofolic acid, THF)	$C_{20}H_{28}N_4O_6$ 445.44		Pale cream-coloured powder, oxidizing in air, unstable to light, particularly in solution (-)-L-Form: $[\alpha]_D^{25} - 16.9^\circ$	Active form of folic acid
5-Formyltetrahydropteroylglutamic acid (citrovorum factor, folinic acid, leucovorin)	$C_{20}H_{28}N_4O_7$ 473.45		Colourless crystals $[\alpha]_D^{25} - 15.1^\circ$ (natural factor) $[\alpha]_D^{25} + 16.7^\circ$ (racemate, synthetic factor)	In micro-organisms. Growth factor for <i>Leuconostoc citrovorum</i> , <i>Lactobacillus casei</i> , <i>Streptococcus faecalis</i> , <i>Lactobacillus arabinosus</i> ; carrier of one-carbon units
5,10-Methylenetetrahydropteroylglutamic acid ('active formaldehyde')	$C_{20}H_{28}N_4O_6$ 457.45		Unstable in acid and neutral media	Carrier of one-carbon units
5-Methyltetrahydropteroylglutamic acid	$C_{20}H_{28}N_4O_6$ 459.47			In serum and liver. Carrier of one-carbon units
4-Aminopteroylglutamic acid (aminopterin)	$C_{20}H_{28}N_4O_6$ 440.42		Yellow needles	Antagonist of folic acid; inhibits cell division

* In accordance with the recommendations of the Commission on Biochemical Nomenclature [Biochim. Biophys. Acta (Amst.), 107, 11 (1965)] the names 'folic acid' and 'folate' should be used only as general designations for compounds of the group or mixtures of such compounds and not for any compound named on the basis of its structural formula.

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- ¹⁸ HERBERT et al., *J. Clin. Invest.*, 41, 1134 (1962).
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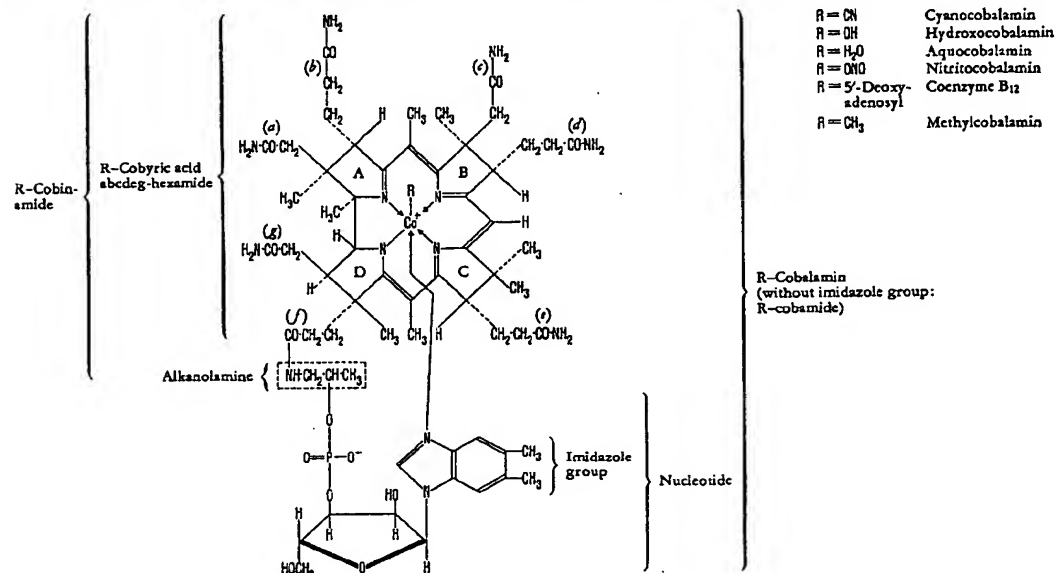
- ²⁴ Food and Nutrition Board, *Recommended Dietary Allowances*, 7th ed., National Academy of Sciences - National Research Council, Publication 1694, Washington, 1968, page 35.
- ²⁵ HERBERT, V., *Arch. intern. Med.*, 110, 649 (1962).
- ²⁶ VELEZ et al., *Amer. J. Clin. Nutr.*, 12, 54 (1963).
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- ²⁸ ALPERIN et al., *Arch. intern. Med.*, 117, 681 (1966); WILLOUGHBY and JEWELL, *Brit. med. J.*, 2, 1568 (1966).
- ²⁹ MATOTH et al., *Amer. J. Clin. Nutr.*, 16, 356 (1965).
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Vitamin B₁₂ group (corrinoids)^{1, 2} (for references see page 485)

Chemistry³

All the complete B₁₂ vitamins contain an α-glycosidic nucleotide. The imidazole nitrogen atom of this nucleotide can become coordinated under suitable conditions with the cobalt atom. In the case of the incomplete types either the alkanolamine and nucleotide

portion, or simply the latter, is lacking; in some cases the nucleotide portion is β-glycosidic, when the imidazole nitrogen atom cannot become co-ordinated with the cobalt atom. The B₁₂ coenzymes correspond to the complete B₁₂ types but in place of the inorganic group they possess an organic group linked directly via a carbon atom to the cobalt atom. The complete and incomplete B₁₂ types fairly stable to light and oxygen are presumably artefacts of the B₁₂ coenzymes.



Names*	Formula and mol. wt.	Physical properties	Occurrence and biological properties
Complete B₁₂ types			
Vitamin B ₁₂ (cyanocobalamin, 5,6-dimethylbenzimidazolylicyanocobamide)	C ₆₃ H ₈₈ N ₁₄ O ₁₄ PCo 1355.40	Red needles, stable on heating several hours at 100 °C. Spectral absorption in water: maxima at 278, 361, 550 nm	Occurs in nature as coenzyme. Can be isolated from animal tissues, many species of bacteria, sewage sludge, activated sludge. Stimulates maturation of erythrocytes in bone marrow; acts as <i>animal protein factor</i> in animals; promotes growth of many micro-organisms
* Trivial names recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry [<i>Biochim. biophys. Acta (Amst.)</i> , 117, 285 (1966)].			

Names*	
Aquocobalamin (vitamin B _{12a} , 5,6-dimethylbenzimidazolylicyanocobamide) Hydroxycobalamin (vitamin B _{12a} , 5,6-dimethylbenzimidazolylicyanocobamide)	Cas: 1347
5-Methylbenzimidazolylicyanocobamide	
Benzimidazolylicyanocobamide	
5-Hydroxybenzimidazolylicyanocobamide (Factor III)	
Pseudovitamin B ₁₂ (adeninecyanocobamide)	
2-Methyladeninecyanocobamide (Factor A)	
Actiocobalamin (Factor B, cyanocobinamide)	
5'-Deoxyadenosylcobalamin (coenzyme B ₁₂)	
Methylcobalamin	

* Trivial names recommended by the Commission on Biochemistry (Biochim. b.

Assay

Microbiological^{1, 4}. With bacteria: *Erct* amide pseudo-vitamin B₁₂, *Lactobacillus* pseudo-vitamin B₁₂. With protozoa: *Ochromonas malha* pseudo-vitamin B₁₂. **Chemical⁵.** Spectrophotometrically or solution; separation of the individual current diffusion, column chromatography. **Isotopic dilution method.** Tagging of ⁵⁷ or ⁶⁰Co.

Vitamin B₁₂ Group (For references see page 485)

483

Recommended Dietary Allowances, 7th ed., National Research Council, Publication 1694.

J. Nutr., 110, 649 (1962).
J. Nutr., 12, 54 (1963).
Nutr., 18, 311 (1966).
ed., 117, 681 (1966); WILLOUGHBY and JEWELL.
J. Nutr., 16, 356 (1965).
J. Nutr., 11, 333 (1960); HELLER and VENGEL, *Med.*
J.; COMPTON and PITCHER, in BARON et al.
Brit. J. Nutr., 14th ed., Churchill, London, 1964, page
N. Amer., 50, 1245 (1966).
Haemat., suppl. *Ser. haemat.*, No. 3, 19 (1965).
ser., 12, 17 (1963).
J. clin. Nutr., 17, 191 (1965).
J. Nutr., 46, 1365 (1962).
J. clin. Nutr., 7, 711 (1959).
550.
med. Rev., 14, 92 (1962).

is lacking; in some cases the nucleotide on the imidazole nitrogen atom cannot be cobalt atom. The B₁₂ coenzymes coexist but in place of the inorganic group is up linked directly via a carbon atom to the and incomplete B₁₂ types fairly stable sumably artefacts of the B₁₂ coenzymes.

R = CN Cyanocobalamin
R = OH Hydroxycobalamin
R = H₂O Aquocobalamin
R = OH Nitrocobalamin
R = 5'-Deoxyadenosyl Coenzyme B₁₂
R = CH₃ Methylcobalamin

Names*	Formula and mol. wt.	Physical properties	Occurrence and biological properties
Aquocobalamin (vitamin B _{12a} , 5,6-dimethylbenzimidazolylaquocobamide) Hydroxycobalamin (vitamin B _{12b} , 5,6-dimethylbenzimidazolylhydroxocobamide)	C ₆₃ H ₉₀ N ₁₂ O ₁₅ PCo 1347.39	Red needles, aquo form in neutral solution, hydroxo form in alkaline solution Spectral absorption in water: maxima at 274, 350, 522 nm	Activity as for cyanocobalamin; depot form in the human body
5-Methylbenzimidazolylcyanocobamide		Red needles	In sewage sludge and activated sludge. Represents two-thirds of the cyanocobalamin activity in pernicious anaemia
Benzimidazolylcyanocobamide		Red needles	In sewage sludge and activated sludge. Represents two-thirds of the cyanocobalamin activity in pernicious anaemia
5-Hydroxybenzimidazolylcyanocobamide (Factor III)		Red needles	Inactivated sludge; weakly active in pernicious anaemia
Pseudovitamin B ₁₂ (adeninecyanocobamide)		Red needles	Inactivated sludge, faeces, stomach contents of ruminants; inactive in pernicious anaemia
2-Methyladeninecyanocobamide (Factor A)		Red needles	Inactivated sludge, faeces, stomach contents of ruminants; very weakly active in pernicious anaemia
<i>Incomplete B₁₂ types</i>			
Actiocobalamin (Factor B, cyanocobinamide)		Amorphous	Inactivated sludge, faeces, stomach contents of ruminants; antagonist of cyanocobalamin in the chick test
<i>B₁₂ coenzymes</i>			
5'-Deoxyadenosylcobalamin (coenzyme B ₁₂)		Orange-yellow platelets, photosensitive	In many species of bacteria, in animal tissues (mainly liver). Biochemically active form of vitamin B ₁₂ . Growth promoting activity for micro-organisms and chicks; activity in pernicious anaemia as for cyanocobalamin; depot action in the human body
Methylcobalamin		Orange-yellow platelets, photosensitive	In animal tissues (liver), blood serum. Coenzyme function

* Trivial names recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry [*Biochim. biophys. Acta (Amst.)*, 117, 285 (1966)].

Assay

Microbiological^{1, 4}. With bacteria: *Escherichia coli* (cobalamins, cobamide pseudo-vitamin B₁₂), *Lactobacillus leichmannii* (cobalamins, pseudo-vitamin B₁₂). With protozoa: *Euglena gracilis* (cobalamins, pseudo-vitamin B₁₂), *Ochromonas malhamensis* (cobalamins only).

*Chemical*⁵. Spectrophotometrically or polarographically in pure solution; separation of the individual compounds by counter-current diffusion, column chromatography or ion exchange.

Isotopic dilution method. Tagging of vitamin B₁₂ with ⁵⁷Co, ⁵⁸Co or ⁶⁰Co.

Unit

No international unit; by weight. 1 μg vitamin B₁₂ = 11 000 LLD (*Lactobacillus lactis* DORNER) Units = 1 USP Unit (liver extract). 1 USP Unit is the daily dose that produces a clinically and haematologically satisfactory response in true pernicious anaemia. From the standpoint of activity, 1000 LLD Units corresponds roughly to 1 ml of a good liver extract.

For International Reference Preparation see page 763.

Occurrence and biological properties

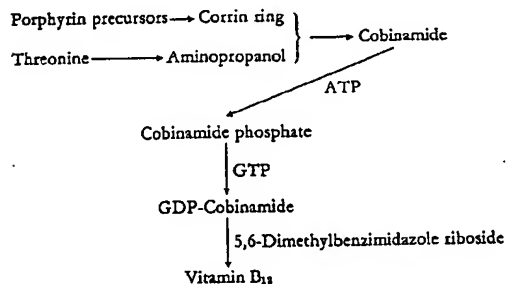
is in nature as coenzyme. Can be isolated from animal tissues, many species of bacteria, sewage sludge, activated sludge. Stimulates maturation of erythrocytes in marrow; acts as animal protein factor in animals; promotes growth of many micro-organisms

of Pure and Applied Chemistry and the International Union of Biochemistry

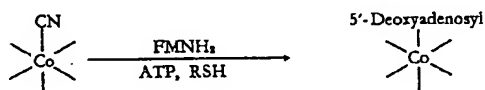
Biogenesis^{6,7}

Vitamin B₁₂ is synthesized by many species of bacteria, in particular the propionibacteria and *Arrobacter aerogenes*. It is possibly also formed in animal tissues⁸.

The biosynthesis is in accordance roughly with the following scheme:



This process results in formation of the coenzyme forms of the vitamin; cyanocobalamin and hydroxocobalamin are probably only artefacts⁹; they can, however, be converted in animal tissues into the coenzymes:



Intake and excretion

In the USA the average daily diet contains 15–30 µg vitamin B₁₂¹⁰, of which perhaps 5 µg is absorbed. According to HEINRICH and WOLFE^{11,12}, of any dose of vitamin B₁₂, 1.5 µg is absorbed in the ileum with the aid of the so-called *intrinsic factor*, a mucoprotein of the gastric juice¹¹; transport through the intestinal wall probably takes place together with the intrinsic factor¹³. In addition, there is passive diffusion through the intestinal wall¹⁴ to an extent that increases logarithmically with increasing size of the dose up to a limiting value of 0.9% of the dose¹². On a normal diet and three meals a day this means that 2–5 µg or more of vitamin B₁₂ is absorbed daily¹⁵ (see the figure below). Cyanocobalamin is rather more easily absorbed than coenzyme B₁₂¹⁶. Some 10–15 µg vitamin B₁₂ is synthesized daily by bacteria in the human large intestine, and about the same quantity is excreted daily in the faeces; whether any of the quantity formed by bacterial synthesis is absorbed is doubtful¹⁷.

The vitamin B₁₂ content of the serum lies in the range 100–900 ng/l (see page 610); here the vitamin is mainly present as methylcobalamin, about 80% being bound to α-globulins (transcobalamin I). Exogenous vitamin B₁₂ becomes bound for a short time to β-globulin (transcobalamin II)¹⁸. The half-life of intravenously administered cyanocobalamin in the serum is about 6 days¹⁹. Small doses of a few microgrammes of vitamin B₁₂ are retained in the body but doses of a few milligrammes are rapidly excreted in the urine. Hydroxocobalamin given parenterally is retained in the body longer than cyanocobalamin or coenzyme B₁₂²⁰. In the tissues the vitamin is probably stored as the coenzyme. The total body stores of the vitamin have been estimated at 2–5 mg (range 1–11 mg)^{1,12,18}. The liver contains about 0.8 mg vitamin B₁₂²⁰ (the biological half-life of the vitamin in the liver is about 12 months¹⁹). 0.1% of the body's stores of vitamin B₁₂ is excreted daily¹⁵. These stores probably suffice to prevent the appearance of clinical deficiency symptoms for 3–8 years^{1,18}. Excretion of the vitamin is almost solely in the bile (see page 655), only very small amounts being found in the urine (0–0.27 µg per day)¹. On SCHILLING's excretion test see pages 289 and 485.

Function^{7,21}

Vitamin B₁₂ acts as coenzyme in va

Enzyme (reaction catalysed in brackets)	C
Methylaspartate mutase (glutamic acid ⇌ β-methylaspartic acid)	5'-Dec cobala
Methylmalonyl-CoA- mutase (methylmalonyl- CoA ⇌ succinyl-CoA)	5'-Dec cobala
Glycol dehydrogenase (ethyleneglycol ⇌ acetaldehyde, 1,2-propane- diol ⇌ propionaldehyde)	5'-Dec cobala
5-Methyltetrahydrofolate homocysteine trans- methylase (Methionine formation)	Methy (prote as inte produ
Ribonucleotide reductase (DNA formation)	5'-Dec cobala
(Thiol oxidation)	Unkn

Inside the cell, vitamin B₁₂ is located in a manner still largely un-ably also lipid and carbohydrate meta the vitamin in the formation of methi in the regeneration of tetrahydrofolic ; B₁₂ plays a very important part in haen reticulocytes) in that together with fol; or indirectly in the formation of deox nucleotides. The significance of the vits is obscure (more is known of this role i is the part it plays in the maturation c growth²².

Requirements

The daily requirement in adults app the absorption of 0.6–1.5 µg¹⁹, but i level is 5 µg²³ (see page 494). In pregn may be higher since the mother's reser the needs of the foetus; the Food and accordingly recommend a daily intake

The best sources of vitamin B₁₂ (in tance) are liver, kidneys, meat and mil present in plants (see pages 499–515), than 30% of the vitamin¹⁶.

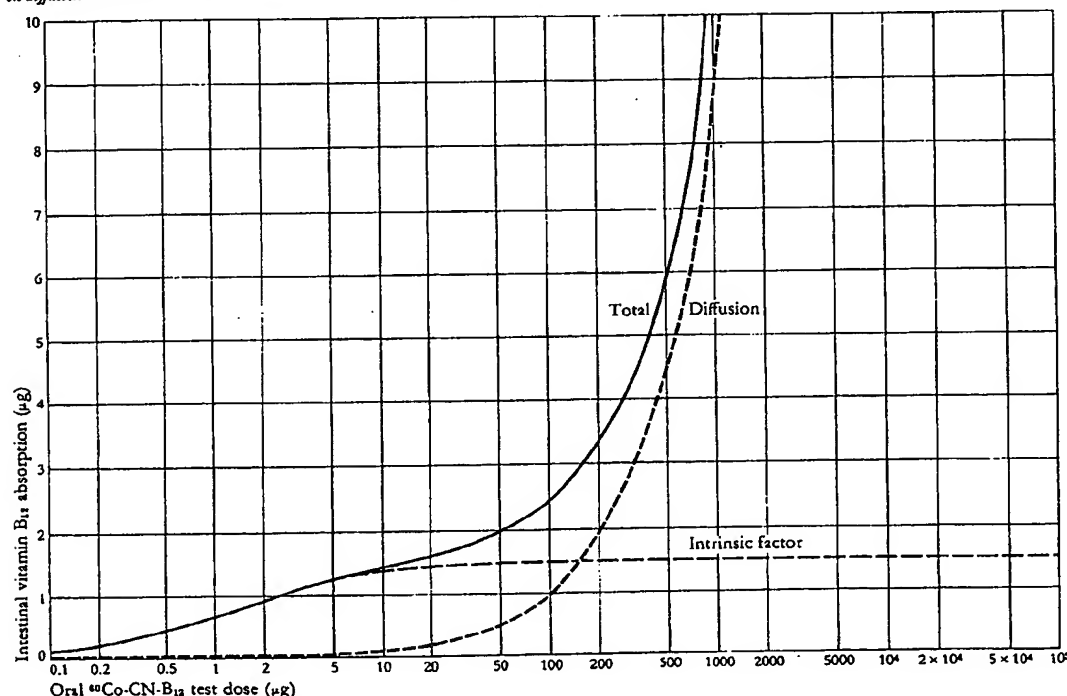
Deficiency²⁷

Vitamin B₁₂ deficiency may arise fro

- Inadequate dietary intake (strict ve
- Absence or inadequacy of intrinsic anaemia, gastrectomy, gastroenteric juice of antibodies to the intrin
- Inadequate ileal absorption, (malal tuberculosis, ileal resection)
- Interference with absorption by ba in blind loops, diverticulosis of th fish tapeworm.

The following are the deficiency sym megaloblastosis of the bone marrow penia, glossitis, morphological change; and (in contrast to folic acid deficiency) the axis cylinders of the spinal-cord ne of the pernicious anaemia is possibly in serum of many patients contains antib leading to atrophy of the gastric muc pernicious anaemia there is absence of a in children secretion of the intrinsic i; probably as a genetic defect – but acid

Distribution of the total intestinal vitamin B₁₂ absorption in healthy persons between absorption dependent on the intrinsic factor and absorption dependent on diffusion¹²



Function^{7, 21}Vitamin B₁₂ acts as coenzyme in various biological reactions:

Enzyme (reaction catalysed in brackets)	Coenzyme	Occurrence
Methylaspartate mutase (glutamic acid \rightleftharpoons β -methylaspartic acid)	5'-Deoxyadenosyl- cobalamin	Bacteria
Methylmalonyl-CoA- mutase (methylmalonyl- CoA \rightleftharpoons succinyl-CoA)	5'-Deoxyadenosyl- cobalamin	Bacteria, animal tissues
Glycol dehydrogenase (ethyleneglycol \rightleftharpoons acetaldehyde, 1,2-propane- diol \rightleftharpoons propionaldehyde)	5'-Deoxyadenosyl- cobalamin	Bacteria
5-Methyltetrahydrofolate homocysteine trans- methylase (Methionine formation)	Methylcobalamin (protein complex) as intermediate product	Bacteria, animal tissues
Ribonucleotide reductase (DNA formation)	5'-Deoxyadenosyl- cobalamin	Bacteria, animal tissues
(Thiol oxidation)	Unknown	Bacteria, animal tissues

Inside the cell, vitamin B₁₂ is located in the mitochondria²² and is involved in a manner still largely unknown in protein and probably also lipid and carbohydrate metabolism. The role played by the vitamin in the formation of methionine is probably important in the regeneration of tetrahydrofolic acid (see page 437). Vitamin B₁₂ plays a very important part in haemopoiesis (stimulation of the reticulocytes) in that together with folic acid it is involved directly or indirectly in the formation of deoxyribonucleotides from ribonucleotides. The significance of the vitamin in normal reproduction is obscure (more is known of this role in animals than in man²³), as is the part it plays in the maturation of the spermatozoa²⁴ and in growth²⁵.

Requirements

The daily requirement in adults appears to be amply covered by the absorption of 0.6–1.5 μg ¹⁸, but a desirable daily absorption level is 5 μg ²⁶ (see page 494). In pregnant women the requirement may be higher since the mother's reserves are largely exhausted by the needs of the foetus; the Food and Nutrition Board (USA)²⁶ accordingly recommend a daily intake of 8 μg (see page 494).

The best sources of vitamin B₁₂ (in decreasing order of importance) are liver, kidneys, meat and milk; only small quantities are present in plants (see pages 499–515). Cooking destroys not more than 30% of the vitamin²⁸.

Deficiency²⁷Vitamin B₁₂ deficiency may arise from the following causes:

- Inadequate dietary intake (strict vegetarians)
- Absence or inadequacy of intrinsic factor secretion (pernicious anaemia, gastrectomy, gastroenterostomy; presence in the gastric juice of antibodies to the intrinsic factor²⁹)
- Inadequate ileal absorption (malabsorption syndrome, ileitis, tuberculosis, ileal resection)
- Interference with absorption by bacteria (intestinal congestion in blind loops, diverticulosis of the small intestine) or by the fish tapeworm.

The following are the deficiency symptoms: macrocytic anaemia, megaloblastosis of the bone marrow, leucopenia, thrombocytopenia, glossitis, morphological changes in the gastrointestinal tract and (in contrast to folic acid deficiency) progressive degeneration of the axis cylinders of the spinal-cord neurones. In adults, the cause of the pernicious anaemia is possibly immunological processes (the serum of many patients contains antibodies to the intrinsic factor) leading to atrophy of the gastric mucosa. Whereas in adults with pernicious anaemia there is absence of acid secretion in the stomach, in children secretion of the intrinsic factor itself may be absent – probably as a genetic defect – but acid secretion normal²⁹.

Biochemical deficiency symptoms of use in diagnosis are the low-red vitamin B₁₂ content of the serum (see page 610) and liver and the very much increased excretion of methylmalonic acid in the urine³⁰. The amount of vitamin B₁₂ absorbed from the diet can be estimated by measurement of the total faecal excretion over a period of 5 or more days, of the excretion in a single faecal sample using a double isotope technique with a nonabsorbable marker, of the hepatic uptake of radioactivity, of the urinary excretion after a flushing dose of untagged vitamin B₁₂, or of the whole-body retention of radioactivity. In malabsorption the test is repeated with intrinsic factor to see if this improves absorption; alternatively vitamin B₁₂ and vitamin B₁₂ intrinsic factor tagged with two different cobalt isotopes can be given simultaneously³¹. SCHILLING's test (0.5 μg tagged vitamin B₁₂ orally followed one hour later by 1000 μg untagged vitamin B₁₂ intramuscularly) gives the following values³²: normal 8–34%, in pernicious anaemia 0–3.5% of the radioactivity in the 24-hour urine.

Treatment

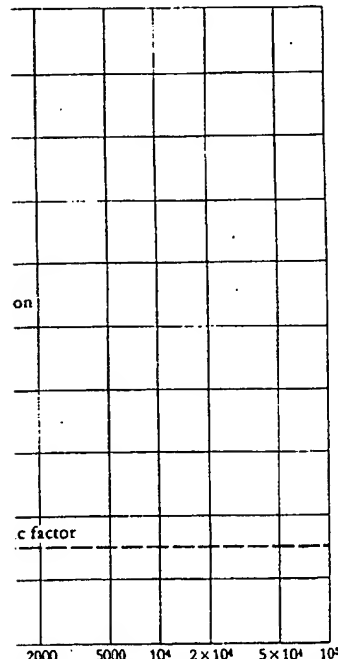
The blood picture in pernicious anaemia shows an improvement with parenteral doses of as little as 0.1 μg vitamin B₁₂ per day³³; doses of 0.5–2.0 μg per day result in complete remission of the symptoms³⁴. The body's reserves can be restored within 10 days by giving 5 doses each of 1000 μg subcutaneously. The usual maintenance dose is 100–400 μg parenterally once a month or 1000 μg orally twice a week³⁵. Oral treatment with vitamin B₁₂ plus intrinsic factor is not recommended since the body eventually develops resistance. Oral treatment with a vitamin B₁₂-peptide complex has been suggested³⁶.

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ally diet contains 15–30 μg vitamin B₁₂¹⁰, absorbed. According to HEINRICH and use of vitamin B₁₂, 1.5 μg is absorbed in a so-called *intrinsic factor*, a mucoprotein port through the intestinal wall probably the intrinsic factor¹². In addition, there is the intestinal wall¹⁴ to an extent that with increasing size of the dose up to a the dose¹². On a normal diet and three 2–5 μg or more of vitamin B₁₂ is absorbed w). Cyanocobalamin is rather more easily absorbed¹⁶. Some 10–15 μg vitamin B₁₂ is syn- in the human large intestine, and about ed daily in the faeces; whether any of the al synthesis is absorbed is doubtful¹⁷. t of the serum lies in the range 100–900 e vitamin is mainly present as methylco- bound to α -globulins (transcobalamin I). comes bound for a short time to β -globu- The half-life of intravenously adminis- he serum is about 6 days¹⁸. Small doses vitamin B₁₂ are retained in the body but s are rapidly excreted in the urine. Hydro- rally is retained in the body longer than yme B₁₂¹⁸. In the tissues the vitamin is nzyme. The total body stores of the ted at 2–5 mg (range 1–11 mg)^{11, 22, 23}. .8 mg vitamin B₁₂²⁰ (the biological half- iver is about 12 months²²). 0.1% of the is excreted daily¹⁸. These stores prob- appearance of clinical deficiency sym- of the vitamin is almost solely in the y small amounts being found in the urine SCHILLING's excretion test see pages 289

n the intrinsic factor and absorption dependent



Biotin¹

Chemistry

For structure and properties of biotin and related compounds see the table below.

Assay

Biological². With *Saccharomyces cerevisiae*, *Lactobacillus casei*, *Lactobacillus arabinosus*, *Neurospora crassa*, etc.; in biological fluids preferably with *Ochromonas danica*.

Chemical. No methods in common use.

Unit

No international unit; by weight. 1 avidine unit = the smallest quantity that completely suppresses the growth-promoting effect in yeast of 1 µg biotin⁴.

Biogenesis^{2, 5}

Biotin is synthesized in plants (particularly in sprouting seeds) and various micro-organisms. Thus *Achromobacter* forms biotin from pimelyl-coenzyme A, cysteine and carbamyl phosphate; pimelyl-coenzyme A is formed from 3 molecules of malonyl-coenzyme A⁶.

The carboxyl group of biotin is covalent and bound to the lysine residue of a protein. Biocytin is formed by the action of a proteinase on protein-bound biotin.

Intake and excretion

Biotin is formed by the intestinal flora in such large quantities that it is excreted in the faeces in an amount 2–5 times greater than the dietary intake⁷. The body appears to be capable of utilizing biotin formed in the intestine but to an unknown extent. The avidine present in raw egg-albumin combines with biotin, thus rendering it useless for the organism.

Biotin has been detected in whole blood and serum (see page 611) and in urine (see page 676). Small quantities are stored in the liver (about 0.2 mg) and in the brain⁸. (blood vessels 3–5 ng/g⁹). In the liver, biotin is mostly bound to protein (in rats 90%⁹). Little is known of the metabolism of biotin. In rats given injections of biotin 16% was found 4 hours later in the liver and 30% was excreted⁸.

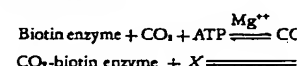
Function⁵

Biotin is essential for warm-blooded animals and some micro-organisms. It acts as coenzyme in CO₂-fixation and transcarboxylation reactions.

Biotin enzymes

Enzyme	Reaction catalysed
Acetyl-CoA-carboxylase	acetyl-CoA + HCC ATP \rightleftharpoons malonyl-CoA + ADP + P
β-Methylcrotonyl-CoA-carboxylase	β-methylcrotonyl-HCO ₃ ⁻ + ATP \rightleftharpoons β-methylglutaconyl-CoA + ADP + P
Propionyl-CoA-carboxylase	propionyl-CoA + HCC ATP \rightleftharpoons methylcrotonyl-CoA + ADP + P
Methylmalonyl-CoA-carboxyl-transferase	methylmalonyl-CoA + pyruvate \rightleftharpoons propionyl-CoA + oxalacetate
Pyruvate carboxylase	pyruvate + HCO ₃ ⁻ \rightleftharpoons oxalacetate + A ¹⁰

The carboxylation reactions catalysed the general form



(where X is for example β-methylcrotonyl, in which the CO₂ is N-carboxybiotin in the table opposite acetyl-coenzyme A, on the other hand, of the biotin itself represents the active as 'diaminobiotin' is here assumed to a

The carboxylation of acetylcoenzyme A point in the biosynthesis of fatty acids an important role in many other reactions only in an indirect manner. Example deamination of aspartate, serine and threonine of serine in animals, reductive carboxylation of phosphoenol pyruvate tryptophan metabolism, purine synthesis carbohydrate metabolism.

Certain biotin homologues (homologues which the sulphur atom is replaced by selenium)

Requirements and deficiency symptoms

The biotin requirement of man is uncertain, deficiency, doses of 150–300 µg per day deficiency symptoms^{12, 13}; this amount diet¹⁴.

Substances rich in biotin are liver, the vitamin is also present in vegetable 498–515).

Biotin deficiency is manifested¹⁵ in pigs, chickens, man, hyperkeratosis (man), loss of hair (rats, mice), loss of appetite, nausea, muscular pain and spontaneous biotin deficiency has been reported in eggs^{17, 18} and also appears to be in

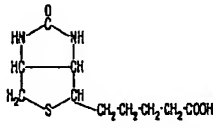
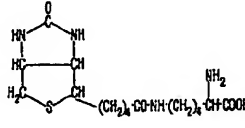
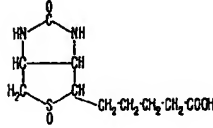
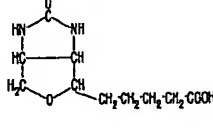
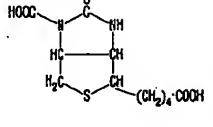
Treatment

Biotin has been used to treat seborrheic children (LEINER's disease)¹⁹, which is of biotin in the breast milk together to persistent diarrhoea.

References

1. SIBBELL and HARRIS (Eds.), *The Vitamin Year*, 1954, page 325; CHOW, B.F., in *Nutrition*, vol. 2, Academic Press, New York, 1954, page 100; R. S. in WOHL and GOODHART (Eds.), *Metabolism*, 3rd ed., Lea & Febiger, Philadelphia, 1960.

Structure and properties of biotin and related compounds

Names	Formula and mol. wt.	Structure	Physical properties	Occurrence	Activity
Biotin, d-biotin	C ₁₀ H ₁₆ N ₂ O ₆ S 244.31		White needles, stable to heat, unstable to acids and alkalis. M. p. 230–232 °C [α] _D ²⁵ + 91° in 0.1-N NaOH	Various micro-organisms, for example yeasts, animal tissues, particularly liver, egg-yolk, plants	Growth factor for many bacteria, protozoa and probably all higher animals
Biocytin, d-biocytin (ε-N-biotinyl-L-lysine)	C ₁₈ H ₂₈ N ₄ O ₆ S 372.49		M. p. 245–252 °C	Yeasts	Growth factor for various micro-organisms
Biotin sulphoxide, d-biotin 1-sulphoxide (AN factor)	C ₁₀ H ₁₆ N ₂ O ₆ S 260.31			Cultures of <i>Aspergillus niger</i> and <i>Phycomyces blakesleeianus</i>	Growth factor for <i>Neurospora crassa</i>
Oxybiotin (oxobiotin)	C ₁₀ H ₁₆ N ₂ O ₆ 228.25		M. p. 205–207 °C		5–30% of activity of biotin ²
1'-N-Carboxybiotin (CO ₂ -biotin)	C ₁₁ H ₁₈ N ₂ O ₇ S 288.33			Unstable intermediate	Active form of CO ₂ in carboxylations

otin is covalent and bound to the lysine
tin is formed by the action of a proteinase

intestinal flora in such large quantities
eces in an amount 2-5 times greater than
body appears to be capable of utilizing
sine but to an unknown extent. The avi-
albumin combines with biotin, thus ren-
rganism.

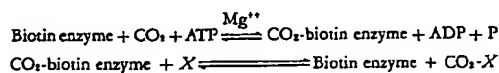
in whole blood and serum (see page 611)
). Small quantities are stored in the liver
rain^{2,6} (blood vessels 3-5 ng/g⁶). In the
und to protein (in rats 90%⁶). Little is
of biotin. In rats given injections of biotin
ater in the liver and 30% was excreted⁷.

arm-blooded animals and some micro-
zyme in CO₂-fixation and transcarboxyl-

Biotin enzymes

Enzyme	Reaction catalysed	Occurrence
Acetyl-CoA-carboxylase	acetyl-CoA + HCO ₃ ⁻ + ATP ⇌ malonyl-CoA + ADP + P	Micro-organisms, chicken liver
β-Methylcrotonyl-CoA-carboxylase	β-methylcrotonyl-CoA + HCO ₃ ⁻ + ATP ⇌ β-methylglutaconyl-CoA + ADP + P	Micro-organisms, rat liver mitochondria
Propionyl-CoA-carboxylase	propionyl-CoA + HCO ₃ ⁻ + ATP ⇌ methylmalonyl-CoA + ADP + P	Swine heart, ox liver mitochondria
Methylmalonyl-CoA-carboxyl-transferase	methylmalonyl-CoA + pyruvate ⇌ propionyl-CoA + oxalacetate	Propionibacteria, skeletal muscle of dogs
Pyruvate carboxylase	pyruvate + HCO ₃ ⁻ + ATP ⇌ oxalacetate + ADP + P	Micro-organisms, liver mitochondria, rabbit kidneys

The carboxylation reactions catalysed by the biotin enzymes have the general form



(where X is for example β-methylcrotonyl-CoA) via a CO₂-biotin enzyme compound in which the CO₂ is linked to biotin (see 1'-N-carboxybiotin in the table opposite)¹⁰. In the carboxylation of acetyl-coenzyme A, on the other hand, the carbamido carbon atom of the biotin itself represents the active CO₂¹¹; a compound known as 'diaminobiotin' is here assumed to arise as intermediate product.

The carboxylation of acetylcoenzyme A is an important starting point in the biosynthesis of fatty acids (see page 424). Biotin plays an important role in many other reactions in which, however, it acts only in an indirect manner. Examples of such reactions are the deamination of aspartate, serine and threonine in bacteria, deamination of serine in animals, reductive carboxylation of pyruvate, carboxylation of phosphoenol pyruvate, carbamylation reactions, tryptophan metabolism, purine synthesis, protein synthesis and carbohydrate metabolism.

Certain biotin homologues (homo-oxybiotin; biotinsulphone, in which the sulphur atom is replaced by a sulphone group) act as anti-vitamins¹².

Requirements and deficiency symptoms

The biotin requirement of man is unknown. In experimental biotin deficiency, doses of 150-300 µg per day sufficed to suppress the deficiency symptoms^{13,14}; this amount is provided by a normal diet¹⁵.

Substances rich in biotin are liver, kidneys, yeast and egg-yolk; the vitamin is also present in vegetables, nuts and cereals (see pages 498-515).

Biotin deficiency is manifested¹⁶ in nervous disturbances (rats, pigs, chickens, man), hyperkeratosis (rats), seborrhoeic dermatitis (man), loss of hair (rats, mice), loss of hair pigment (rats, mice). Experimental biotin deficiency in man results in lethargy, loss of appetite, nausea, muscular pain and localized paresthesia¹⁴. Spontaneous biotin deficiency has been reported following a diet rich in raw eggs^{17,18} and also appears to be associated with liver cirrhosis¹⁹.

Treatment

Biotin has been used to treat seborrhoeic dermatitis in young children (LEINER's disease)²⁰, which is possibly due to a deficiency of biotin in the breast milk together with loss of the vitamin due to persistent diarrhoea.

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19. GAUTIER et al., *Int. Z. Vitaminforsch.*, 28, 61 (1957); NISSEN, A., *J. Pediatr.*, 51, 537 (1957).

Pantothenic acid¹ (for references see page 489)

Chemistry

For structure and properties of pantothenic acid and related compounds see the table on page 488.

Assay

Biological. Growth test on chicks; microbiologically² with *Lactobacillus casei* or *L. plantarum* (*L. arabinosus*).

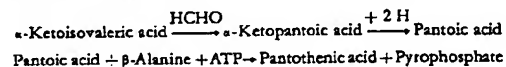
*Chemical*³. After hydrolysis by colorimetric determination of the β-alanine.

Unit

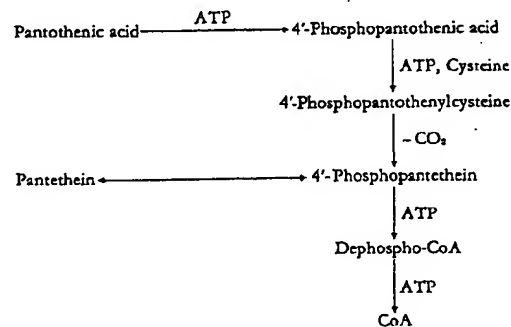
No international unit; by weight. 1 LIPMANN unit of coenzyme A = 2.4 µg of the pure compound (corresponding to 0.7 µg pantothenic acid)⁴.

Biogenesis⁵

Pantothenic acid is formed by bacteria (e.g., *Escherichia coli*, *Bacterium linum*) from α-ketoisovaleric acid by addition of a one-carbon unit. The formation of pantothenic acid from pantoic acid and β-alanine is mediated by ATP (for instance in *Escherichia coli* and *Brucella abortus*) as follows:



In animal tissues no pantothenic acid is formed; the vitamin is, however, incorporated in coenzyme A in both micro-organisms and animal tissues (for instance liver):



Intake and excretion

A 2500 kcal diet contains about 4-12 mg of free and 9-20 mg total pantothenic acid⁷. The vitamin is also synthesized in the human

Occurrence	Activity
Various micro-organisms, for example yeasts, animal tissues, particularly liver, egg-yolk, plants	Growth factor for many bacteria, protozoa and probably all higher animals
Yeasts	Growth factor for various micro-organisms
Cultures of <i>Aspergillus niger</i> and <i>Phycomyces blakesleeanus</i>	Growth factor for <i>Neurospora crassa</i>
	5-30% of activity of biotin ²
Unstable intermediate	Active form of CO ₂ in carboxylations

Structure and properties of pantothenic acid and related compounds

Compound	Formula and mol. wt.	Structure	Physical properties	Occurrence and activity
Pantothenic acid (D-[+]-N-[α,γ-dihydroxy-β-dimethylbutyryl]-β-alanine, chick antidermatitis factor)	C ₉ H ₁₇ NO ₅ 219.24		Yellow oil, unstable to heat, acids and alkalis [α] _D ²⁰ + 37.5° Calcium salt: white crystals, stable to heat	Widely distributed in plants and animals. Growth factor for yeasts, many other micro-organisms and all higher animals; component of coenzyme A
Pantothenyl alcohol (panthenol, N-pantooyl-3-propanolamine)	C ₈ H ₁₅ NO ₄ 205.26		Viscous liquid [α] _D ²⁰ + 29.5°	Synthetic. Shows 86% of activity of pantothenic acid in the chick test ²
Pantethcin (N-pantothenyl-β-aminoethanethiol)	C ₁₁ H ₁₉ N ₃ O ₄ S 278.37		Amorphous powder soluble in water	Growth factor for <i>Lactobacillus bulgaricus</i>
Coenzyme A (CoA)	C ₂₁ H ₃₈ N ₇ O ₁₆ P ₃ S 767.54	See page 345	Colourless powder soluble in water	Widely distributed in micro-organisms, plants and animals. See also text and page 345

intestine, though it is not known whether the body makes use of this source. The pantothenic acid content of whole blood is ca. 0.2–2 mg/l (see page 611), that of the spinal fluid about the same (see page 639). Urinary excretion of the vitamin amounts to 0.76–4.1 mg/l³ (see also page 676). Four hours after giving a test dose of the vitamin there is a sharp rise in the blood and urine levels⁴. In blood and spinal fluid pantothenic acid is in the conjugated form, whereas in the urine it is in the free form⁵. The excretion in the faeces is very variable and depends on the nature of the diet.

Coenzyme A is not present in the circulating blood and appears to pass the cell membrane only with difficulty. It is probably formed within the cell when required. Coenzyme A occurs mainly in the following organs (in order of decreasing concentration): liver, adrenals, kidneys, brain, heart, testes. The pantothenic acid content of the human liver amounts to 28 mg, mainly as coenzyme A⁶.

Function

The importance of pantothenic acid in metabolism is explained by its presence in coenzyme A. In the form of acetyl-coenzyme A ('active acetic acid') it is responsible for transport of two-carbon and other acyl groups (see page 345). Coenzyme A is involved in the following reactions: formation of citrate from oxalacetate and acetate (pages 390 and 424), oxidation of pyruvate (page 391), oxidation of α-ketoglutarate (page 390), oxidation and synthesis of fatty acids (pages 391 and 424), synthesis of triglycerides (page 426), phospholipids (page 425) and cholesterol (page 426), acetylation of amines (page 444), choline (page 434) and glucosamine (see page 424). Pantothenic acid plays an important part in the activity of the adrenal cortex¹⁰ since the corticosteroids are formed from the cholesterol synthesized with the participation of coenzyme A (page 429).

Requirements and deficiency symptoms

The human pantothenic acid requirement is unknown, but in children and adults it is probably met by an intake of 5–10 mg per day¹¹, an amount normally present in the diet. The requirements of children are lower in accordance with their smaller intake of calories¹².

Pantothenic acid is present in almost all vegetables, cereals and animal foods. Good sources of the vitamin are yeast, liver, kidneys, heart (see pages 499–515) and particularly the jelly queen bees are fed on (royal jelly), containing 110–320 μg/g¹³.

Pantothenic acid is so widely distributed in foods that deficiency is practically unknown in man. Deficiency symptoms in animals are degeneration of the neuromuscular structures and adrenal insufficiency, and death may ensue. An experimental deficiency has been produced in man by means of a diet low in the vitamin together with doses of the antagonist N-methylpantothenic acid¹⁴, with the following symptoms: mild fatigue, headache, sleeplessness, nausea, epigastric pain, paraesthesia of the limbs, muscle spasms and co-ordination disturbances; other signs were absence of the eosinophile reaction of the blood to ACTH and an increased sensitivity to insulin. The pantothenic acid deficiency was evidenced by the reduced acetylating capacity of the blood after administration of sulphanilamide or para-aminobenzoic acid.

Treatment

The so-called 'burning feet' syndrome has been treated with pantothenic acid¹⁵, though it is not certain that this syndrome is solely due to pantothenic acid deficiency. Pantothenic acid has been reported to be effective against the neurotoxicity of streptomycin¹⁶; its value in the treatment of emotional disturbances¹⁷, diabetic neuropathy¹⁷, skin diseases¹⁷ and paralytic ileus¹⁸ remains uncertain.

References ('Pantothenic acid', pages 487–

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Ascorbic acid¹⁻³ (for references see p

Chemistry

Compound	Formula and mol. wt.
L-Ascorbic acid (vitamin C)	C ₆ H ₈ O ₆ 176.13
Dehydroascorbic acid	C ₆ H ₆ O ₆ 174.11
2-Keto-L-gulonic acid	C ₆ H ₁₀ O ₇ 194.14

Assay

*Biological*⁴. In guinea-pigs either by structure or by means of the prevention of scurvy.

*Chemical*⁵. Polarographically; by ability of ascorbic acid to reduce 2,6-N-bromosuccinimide (dehydroascorbic acid) to 2,6-dibromosuccinimide, with, for instance, H₂S or allyl using the 2,4-dinitrophenylhydrazide and 2,3-diketone-L-gulonic acid, or concentrated sulphuric acid (ascorbic acid) with, for instance, bromine or acetic acid extracted from biological material solution. Ascorbic acid and dehydroascorbic acid are separated by chromatography.

Unit

No international unit; by weight. T (= 50 μg L-ascorbic acid) is now obsolete.

Biogenesis⁶

Ascorbic acid is synthesized by all except the primates, guinea-pigs, the Indian fruit bat (*Pteropus*) and the Indian fruit bat (*Pteropus*), entirely not formed by micro-organisms, necessary for their growth. Biogenesis takes

Ascorbic Acid

489

eties	Occurrence and activity
stable nd	Widely distributed in plants and animals. Growth factor for yeasts, many other micro-organisms and all higher animals; component of coenzyme A
white to	Synthetic. Shows 86% of activity of pantothenic acid in the chick test ²
owder cr	Growth factor for <i>Lactobacillus bulgaricus</i>
water cr	Widely distributed in micro-organisms, plants and animals. See also text and page 345

Symptoms

Requirement is unknown, but is probably met by an intake of 5-10 mg per cent in the diet. The requirements of mice with their smaller intake of calories

in almost all vegetables, cereals and fruits; the vitamin is yeast, liver, kidneys, particularly the jelly queen bees are 110-320 µg/g¹².

Widely distributed in foods that deficiency in. Deficiency symptoms in animals are muscular structures and adrenal insufficiency. An experimental deficiency has been obtained by a diet low in the vitamin together with 2-methylpantothenic acid¹⁴, with the signs, headache, sleeplessness, nausea, of the limbs, muscle spasms and other signs were absence of the corticosteroids ACTH and an increased sensitivity to deficiency was evidenced by the reticulation of blood after administration of sulfinic acid.

et' syndrome has been treated with it is not certain that this syndrome is deficiency. Pantothenic acid has been shown to be neurotoxicity of streptomycin¹⁶; notional disturbances¹⁷, diabetic neuropathic ileus¹⁸ remains uncertain.

References ('Pantothenic acid', pages 487-488)

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Ascorbic acid¹⁻³ (for references see page 491)

Chemistry

Compound	Formula and mol. wt.	Structure	Physical properties	Occurrence and activity
L-Ascorbic acid (vitamin C)	C ₆ H ₈ O ₆ 176.13		White crystals with an acid taste, soluble in water, rather insoluble in ethanol, sensitive to light, atmospheric oxygen and some heavy metals; strong reducing action M.p. 192 °C. [α] _D +23°	Probably present in all higher plants, particularly cabbage, citrus fruits, hawthorn berries; in small amounts in animal tissues. Antiscorbutic activity
Dehydroascorbic acid	C ₆ H ₆ O ₄ 174.11		White crystals, soluble in water; readily hydrolysed to 2,3-diketo-L-gulonic acid M.p. 225 °C	Present with ascorbic acid in plants. Antiscorbutic activity
2-Keto-L-gulonic acid	C ₆ H ₁₀ O ₇ 194.14		White crystals M.p. 171 °C	Precursor of ascorbic acid. No antiscorbutic activity

Assay

Biological⁴. In guinea-pigs either by histological study of tooth structure or by means of the preventive or curative growth test.

Chemical⁵. Polarographically; by mass analysis based on the ability of ascorbic acid to reduce 2,6-dichlorophenolindophenol or N-bromosuccinimide (dehydroascorbic acid must be reduced beforehand with, for instance, H₂S or homocysteine); photometrically using the 2,4-dinitrophenylhydrazones of dehydroascorbic acid and 2,3-diketo-L-gulonic acid, which give a red solution in concentrated sulphuric acid (ascorbic acid must be oxidized beforehand with, for instance, bromine or active charcoal). The vitamin is best extracted from biological materials with metaphosphoric acid solution. Ascorbic acid and dehydroascorbic acid can be separated from one another and from other substances by paper chromatography.

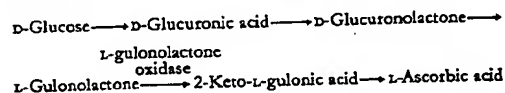
Unit

No international unit; by weight. The former International Unit (= 50 µg L-ascorbic acid) is now obsolete.

Biogenesis⁶

Ascorbic acid is synthesized by higher plants and all animals except the primates, guinea-pigs, the red-vented bulbul (*Pycnonotus cafer*) and the Indian fruit bat (*Pteropus medius*). The vitamin is apparently not formed by micro-organisms, and is presumably not necessary for their growth. Biogenesis takes place in the following steps

(there is some doubt as to whether these are valid for plants):



In most animals ascorbic acid is synthesized in the liver, but in birds, reptiles and amphibians in the kidneys. Animals that do not form ascorbic acid do not possess the enzyme L-gulonolactone oxidase.

Intake and excretion

In the USA the daily intake of ascorbic acid is about 120 mg, half of which arises from citrus fruits and tomatoes⁷. In the gastrointestinal tract ascorbic acid is absorbed in the same way as glucose and other carbohydrates⁸. Even with high intakes of ascorbic acid less than 10 mg of the vitamin is excreted per day in the faeces⁹. Equilibrium between ascorbic acid absorbed from the diet and that present in the tissues is reached in less than 4 hours. Ascorbic acid probably passes the cell membranes in the more lipid-soluble dehydroascorbic acid form, which is then again reduced to ascorbic acid in the cell¹⁰. When the tissues are saturated with ascorbic acid the overall body concentration of the vitamin is 50 mg per kg¹¹. The ascorbic acid contents (mg/kg) of various tissues in human adults are as follows¹²: brain 150, pituitary 150, lens of the

eye 250, adrenals 400, pancreas 150, liver 150, kidneys 50, heart muscle 50. Tissue levels of ascorbic acid are highest at birth and greatly reduced in old age^{1,2}. The saturation level in leucocytes is 270–300 mg ascorbic acid per kg^{1,3}; high doses of ascorbic acid have been reported to increase this concentration up to 600 mg per kg^{1,3}. The whole blood and plasma levels (see page 611) depend on the degree of tissue saturation and on the dietary intake. High doses of ascorbic acid cause the plasma level to rise rapidly, possibly to 40 mg per litre; the normal plasma level is below 14 mg per litre, since at this concentration the threshold value for the kidneys is reached with a consequent steep rise in the ascorbic acid clearance^{3,11}. In the plasma about 20% of the total ascorbic acid is in the form of dehydroascorbic acid¹⁴ (page 611). Ascorbic acid also occurs in the aqueous humour of the eye (50–295 mg/l¹⁵), in gastric juice (see page 650) and in the synovial fluid (see page 642).

Dependence of ascorbic acid levels in serum and leucocytes on intake^{2,3,11}

Daily intake (mg)	Serum (mg/l)	Leucocytes (mg/kg)	Tissue saturation (%)	Percentage of a test dose in the urine [*]
<10	<2	<120	0–40	<5
10–20	~2	~120	~40	<15
30–100	4–10	150–200	>50	20–60
>100	10–14	270–300	→100	60–80

* The usual loading tests consist either of giving 10 mg ascorbic acid orally per kg body weight followed by determination of ascorbic acid in the 24-hour urine, or of giving 100 mg ascorbic acid intravenously followed by determination of ascorbic acid in the 3-hour urine.

With an ascorbic acid concentration of 20 mg per kg body weight the daily turnover is about 1 mg per kg, corresponding to a half-life of 16 days¹⁶. In urine ascorbic acid appears mainly unchanged (see page 676), but part is hydrolysed to diketogulonic acid and appears finally as oxalic acid⁷. Other metabolites are L-xylonic and L-lyxonic acids, which arise by decarboxylation of ascorbic acid¹⁷. Radioactive CO₂ has been detected in expired air after giving doses of tagged ascorbic acid¹⁸. The ascorbic acid content of breast milk (page 689) depends largely on the dietary intake.

Function

Ascorbic acid and dehydroascorbic acid form a redox system with 'semidehydroascorbic acid' as highly reactive intermediate. The latter arises by the loss of a single electron by ascorbic acid or by the acceptance of a single electron by dehydroascorbic acid¹⁹. Almost all metabolic processes the disturbance of which gives rise to scurvy involve reactions in which ascorbic acid is oxidized. Of particular importance are the hydroxylation reactions dependent on ascorbic acid which require molecular oxygen.

The disturbances in the formation of connective tissue that appear in ascorbic acid deficiency are a result of failure of the ascorbic acid-dependent hydroxylation of proline to hydroxyproline, a component of collagen. The formation and maintenance of collagen is dependent on a normal ascorbic acid level²⁰. Synthesis of collagen in human skin-tissue cultures is promoted by ascorbic acid²⁰. In guinea-pigs with ascorbic acid deficiency the formation of hydroxyproline in granulation tissue commences only after ascorbic acid has been administered²¹.

Another hydroxylation reaction dependent on ascorbic acid is the hydroxylation of the side chain of dopamine to noradrenaline; the enzyme catalysing this reaction has been found in the microsomes of beef adrenal cortex²². Ascorbic acid is probably involved also in the hydroxylations occurring in steroid synthesis in the adrenals, but little is known of this role^{22,23}.

Ascorbic acid is also concerned in tyrosine metabolism as reducing agent, although here it does not participate in a hydroxylation reaction. Its probable effect is that of protecting the enzyme para-hydroxyphenylpyruvic acid hydroxylase from inhibition by its substrate²⁴. Ascorbic acid also plays a role as electron donor in the conversion of folic acid into tetrahydrofolic acid. This connection between ascorbic acid and tetrahydrofolic acid may be responsible for the appearance of macrocytic anaemia in scurvy¹⁰. The hypochromic anaemia of scurvy, on the other hand, is more likely to be due to an effect of ascorbic acid on iron metabolism, since the vitamin is necessary for the incorporation of iron into ferritin²⁵.

In contrast to the reactions dependent on ascorbic acid, the hydroxylation of tryptophan to 5-hydroxytryptophan, the precursor of serotonin, is mediated by dehydroascorbic acid, which is thereby reduced to ascorbic acid. The enzyme catalysing this reaction is dependent on copper ions for its activity and occurs mainly in the tissues of the small intestine²⁶. The regeneration of ascorbic acid from dehydroascorbic acid in the tissues plays an important metabolic role. It takes place with the formation of semidehydroascorbic acid from ascorbic acid and dehydroascorbic acid, whereby an enzyme system present in the animal cell transfers electrons from NADH₂ to semidehydroascorbic acid and so regenerates ascorbic acid¹⁰.

In the animal organism ascorbic acid seems to have a protective action against deficiencies of other vitamins (thiamine, riboflavin, pantothenic acid, biotin, folic acid, vitamin E, vitamin A); the relationships between these vitamins and ascorbic acid are, however, obscure²⁷.

Requirements and deficiency symptoms

The minimum intake of ascorbic acid required to prevent scurvy in infants is about 10 mg per day, in adults a little under 10 mg per day²⁸. A daily intake of 30–40 mg results in moderate saturation of the tissues, one of 60–100 mg in almost complete saturation²⁸. The recommended daily intakes of ascorbic acid in various countries are as follows: England²²: infants 15 mg, children 20–30 mg, adults 30 mg; USA²⁹: infants 35 mg, children 40–80 mg, adults 60 mg; Western Germany³⁰: infants 30–35 mg, children 40–50 mg, adults 75 mg; Canada³¹: children 20–30 mg, adults 30 mg; Holland³²: children 35–75 mg, adults 50 mg; Japan³³: children 30–90 mg, adults 60–65 mg. For the recommendations of the Food and Nutrition Board (USA)²⁹ for pregnant and lactating women see the table on page 494. Workers in very cold climates should have an intake of 150–250 mg per day³⁴.

Good sources of ascorbic acid are cabbage, spinach, paprikas, citrus fruits, tomatoes, strawberries, red currants and liver (see pages 499–515). In vegetables the ascorbic acid falls rapidly during withering. Potatoes are an important source of ascorbic acid but the concentration decreases by up to 80% in winter storage. Fresh cow's milk contains up to 25 mg per litre; this is markedly reduced by pasteurization or boiling.

The most important symptoms of ascorbic acid deficiency are a marked tendency to bleeding with the appearance of extensive patches of haemorrhage under the skin and in the gums, muscles, fatty tissues and internal organs. Other symptoms are impairment of connective tissue formation with changes in bone structure and growth, defective tooth formation and fissuring and roughening of the skin; there are also often disturbances of iron absorption and anaemia. In infants ascorbic acid deficiency (Möller-Barlow disease) is manifested mainly in the bones, which show a zone of destroyed bone extending over the margin of the metaphysis into the soft tissues, as well as by subperiosteal bleeding, particularly in the metaphyseal zones of the long bones.

On a diet free of ascorbic acid the ascorbic acid content of the plasma falls after 40 days to less than 1 mg per litre, while after 120 days that of the leucocytes is almost zero². At this time there is also enlargement and keratosis of the hair follicles, which in the succeeding 40 days gradually develop haemorrhages and the characteristic signs of scurvy; changes in the gingiva appear after 180 days³⁵.

An inadequate intake of ascorbic acid is best recognized by the lowered plasma level, whereas a severe deficiency of the vitamin is characterized by the low ascorbic acid concentration of the leucocytes (<120 mg/kg; see the table above). Various tests are available for measuring the extent to which ascorbic acid is provided by the diet; they depend on the degree of tissue saturation effected by a test dose of the vitamin as indicated by the plasma level and urinary excretion.

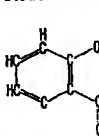
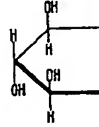

Treatment

In infants, doses of 20 mg ascorbic acid per day, for instance in the form of orange juice, are ample to prevent the appearance of scurvy; in infants with scurvy 25 mg should be given 4 times a day, in adults with the disease 100 mg 5 or 6 times a day²⁸. In patients who have undergone extensive surgery 150–300 mg ascorbic acid per day is sufficient to produce adequate saturation of the tissues³⁷. The reducing properties of ascorbic acid can be utilized in the treatment of methaemoglobinemia and to promote the absorption of orally administered iron³⁸. Russian workers have reported success in the treatment of coronary diseases with ascorbic acid³⁹.

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Substances with vitamin-like

Compound	Formula physica
Bioflavonoids ² (vitamin P group, citrin)	Structure of  Substances and eriodict this group
Mesoinositol ⁴ (myoinositol)	 C ₆ H ₁₂ O ₆ Mol. wt. 180 M.p. 225–2°
Carnitine ⁶ (β-hydroxy- γ-butyro- betaine, vitamin B ₁₂)	 C ₇ H ₁₅ NO ₃ Mol. wt. 166 [α] _D –20.9

* According to BERLIN¹ these are the form of enzymes.

ms dependent on ascorbic acid, the 1 to 5-hydroxytryptophan, the pre- by dehydroascorbic acid, which is acid. The enzyme catalysing this reac- for its activity and occurs mainly stine²⁶. The regeneration of ascorbic cid in the tissues plays an important with the formation of semidehydro- id and dehydroascorbic acid, whereby he animal cell transfers electrons from rbic acid and so regenerates ascorbic

corbic acid seems to have a protective other vitamins (thiamine, riboflavin, ic acid, vitamin E, vitamin A); the vitamins and ascorbic acid are, how-

ncy symptoms

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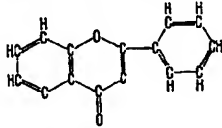
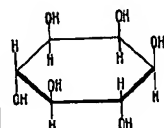
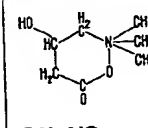
corbic acid is best recognized by the as a severe deficiency of the vitamin is orbic acid concentration of the leuco- :table above). Various tests are avail- to which ascorbic acid is provided by degree of tissue saturation effected by a dicated by the plasma level and urinary

; ascorbic acid per day, for instance in e ample to prevent the appearance of y 25 mg should be given 4 times a day, 0 mg 5 or 6 times a day²⁸. In patients ive surgery 150-300 mg ascorbic acid ce adequate saturation of the tissues³⁷. corbic acid can be utilized in the treat- nia and to promote the absorption of Russian workers have reported success diseases with ascorbic acid³⁹.

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Substances with vitamin-like action (vitaminoids)*

Compound	Formula, mol. wt. and physical properties	Occurrence	Function	Requirements and deficiency symptoms
Bioflavonoids ² (vitamin P group, citrin)	Structure of the flavones:  Substances like hesperidine and eriodictiol also belong to this group	Widely distributed in plants, particularly in fruits (e.g., lemons and black currants)	Biological function obscure. Views on pharmacological activity vary. Substances increasing capillary resistance probably possess antihistamine and antihyaluronidase activity ²	
Mesoinositol ⁴ (myoinositol)	 C ₆ H ₁₂ O ₆ Mol. wt. 180.16 M.p. 225-227 °C	Probable component of all living cells. Component of phospholipids in leaves, seeds and animal tissues (particularly heart, brain and skeletal muscle); present as hexaphosphate (phytic acid) in plants	In the phospholipid form involved in cation transport through the cell membranes, in stimulation of nerves and in metabolism of the mitochondria ⁵	Growth factor for yeasts and many kinds of animal cells in tissue culture. Synthesized in the animal organism. Significance in human nutrition obscure. Intake about 1 g per day
Carnitine ⁶ (β-hydroxy-γ-butyrobetaine, vitamin B ₁₂)	 C ₇ H ₁₅ NO ₃ Mol. wt. 161.20 [α] _D -20.9°	In all animal tissues (skeletal muscle 1, heart 0.6, kidneys 0.4, liver 0.3 mg/gramme dry substance); small amounts in blood, milk, plants and micro-organisms	Involved in intracellular fat metabolism in the form of acylcarnitines by (a) transporting acetyl-coenzyme A and acetoacetyl-coenzyme A from the mitochondria to the site of synthesis of long-chain fatty acids outside the mitochondria ⁷ ; (b) transporting activated long-chain acyl groups from the cytoplasm to the mitochondria, where long-chain fatty acids are oxidized ⁸	Growth factor for some insects, for instance mealworm, <i>Tenebrio molitor</i> and some bacterial species. Vertebrates can synthesize carnitine

* According to BRASIN⁷ these are substances that must be regarded as essential dietary components for many living organisms but that do not function in the form of enzymes.

Compound	Formula, mol. wt. and physical properties	Occurrence	Function	Requirements and deficiency symptoms
Choline ⁹ (β-hydroxyethyltrimethylammonium hydroxide)	$\text{HOCH}_2\text{CH}_2\text{N}^+(\text{CH}_3)_3$ $\text{C}_5\text{H}_{15}\text{NO}_2$ Mol. wt. 121.18 M. p. 180 °C (decomp.)	Component of lecithin, plasmalogens, sphingomyelin and acylcholines. Widely distributed in plants and animals (egg-yolk 17, meat 6, cereals 1mg per gramme). See also pages 394 and 434	Methyl-group donor, replaceable by other sources of labile methyl groups or when synthesized from the latter in the body. Involved in transport of fatty acids from the liver to peripheral fat stores	Growth factor for various micro-organisms, also for rats, chickens and turkeys. Choline deficiency in animals results in injury to the liver and kidneys. Significance in human nutrition obscure. Intake about 0.5–0.9 g per day, an adequate amount in the light of the known requirement of 0.1–0.15 g per 100 g of food ¹⁰
α-Lipoic acid ¹¹ (thioctic acid)	$\text{H}_2\text{C}(\text{S})\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{COOH}$ $\text{C}_8\text{H}_{14}\text{O}_2\text{S}_2$ Mol. wt. 206.33 M. p. 48 °C	In small amounts in vegetable and animal tissues, particularly yeasts and liver	Involved in the oxidative decarboxylation of pyruvic and α-ketoglutaric acids (see pages 390 and 391)	Growth factor for certain bacterial and protozoal species. Probably of no importance in nutrition of higher animals
Essential fatty acids ¹² (vitamin F)	Linoleic and arachidonic acids (see page 370) Unsaturated fatty acids and related compounds such as the corresponding alcohols not synthesized by the body in adequate quantity but necessary for metabolism and growth	In the diet, particularly linoleic acid (plant oils, animal fats) and arachidonic acid (animal fats). Also active are linoleic acid precursors such as linoleyl alcohol and cis-2-octenic acid ¹³ ; linolenic acid is almost inactive. Arachidonic acid is synthesized in the animal body from linoleic acid	Involved in formation of the cell membranes and possibly transport of fatty acids. Structurally essential components of phospholipids and precursors of prostaglandins. Play a role in metabolism of the mitochondria. Many polyene fatty acids such as linoleic and arachidonic acids but also others with no effect on growth are capable of lowering the serum cholesterol level	Deficiency symptoms: in young rats cessation of growth and eczema; in infants eczema ¹⁴ . The increased water intake in rats with deficiency can be utilized to measure activity ¹⁵ . In deficiency the triene acid content of the serum is increased (formation of 5,8,11- and 7,10,13-eicosatrienic acids), the tetraene acid content (arachidonic acid) lowered ^{16,17} . The minimum essential fatty acid requirement in man is about 1–2% of the calorie intake ^{18,19} , or 1.2–2.4 g linoleic acid per 1000 kcal dietary intake. Optimal requirement in infancy: 4% of the calorie intake in the form of linoleic acid ¹⁹ . The tocopherol requirement rises with increasing intake of essential fatty acids (see page 466)

* See footnote, page 491.

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A nutritional standard is a stated nutrients (usually the average daily for a person representative of the which the standard applies¹. Term and nutritional allowance are also regarded as synonymous. The minimum, average or desirable. Si define an individual's optimum requirement, other methods of evaluating come necessary. In USA, the reco

Recommended daily allowances of calor

Age
0–3 months**
4–6 months**
7–12 months
1 year
2 years
3 years
4–6 years
7–9 years
10–12 years
13–15 (boys)
(girls)
16–19 (boys)
(girls)
Adults (men)
(women)

* The amounts given do not cover : infections, malabsorption, metabolic conditions, etc. They are also applic calories and all other nutrients are ful

** For infants from 0 to 6 months, i well-nourished mother is the best way for these vitamins.

Daily protein requirements (FAO/W.

Age
Infants ¹
0–3 months
3–6 months
6–9 months
9–12 months
Juveniles
1–3 years
4–6 years
7–9 years
10–12 years
13–15 years
16–19 years
Adults ¹¹

* The composition of the Reference proteins of eggs, milk and meat all h intake of proteins of lower biologic increased.

** This range is based on the expected upper level is likely to cover the requ and can thus be regarded as a practica

¹ In terms of either breast milk or co¹ During the second and third trimest be added, during lactation 15 g per d

Calories¹

The body requires food energy f of body tissues, physical activities tenance of thermal balance. The e levels of activity are given in the ti

(For references see page 497)

a	Requirements and deficiency symptoms
donor, re- sources groups or ed from body. In- sport of the liver stores	Growth factor for various micro-organisms, also for rats, chickens and turkeys. Choline deficiency in animals results in injury to the liver and kidneys. Significance in human nutrition obscure. Intake about 0.5-0.9 g per day, an adequate amount in the light of the known requirement of 0.1-0.15 g per 100 g of food ¹⁰
oxidative of pyru- oglutaric 390 and	Growth factor for certain bacterial and protozoal species. Probably of no importance in nutrition of higher animals
ation of nes and of fatty y essen- of phos- cursors . Play a n of the y poly- as lino- ic acids with no are ca- the se- vel	Deficiency symptoms: in young rats cessation of growth and eczema ¹⁴ . The increased water intake in rats with deficiency can be utilized to measure activity ¹⁵ . In deficiency the triene acid content of the serum is increased (formation of 5,8,11- and 7,10,13-eicosatrienic acids), the tetraene acid content (arachidonic acid) lowered ^{16,17} . The minimum essential fatty acid requirement in man is about 1-2% of the calorie intake ^{18,19} , or 1.2-2.4 g linoleic acid per 1000 kcal dietary intake. Optimal requirement in infancy: 4% of the calorie intake in the form of linoleic acid ²⁰ . The tocopherol requirement rises with increasing intake of essential fatty acids (see page 466)

1, 285 (1964); WITTELS and BRESSLER,

1 GOODHART (Eds.), *Modern Nutrition in*
& Febiger, Philadelphia, 1964, page 453.
p. *World Health Org. tech. Rep. Ser.*, No. 36210. 1 (1962).
11. 41, 1 (1961).
12. 7 (1962).
13. 1958).
14. *Nutrition*, 25, 62 (1953).
15. 178, 930 (1961).
16. 24, 1 (1966).
17. 14, 70 (1964).
18. 158).

A nutritional standard is a statement of the amounts of certain nutrients (usually the average daily amounts) regarded as necessary for a person representative of the category of the population to which the standard applies¹. Terms such as nutritional requirement and nutritional allowance are also used but must not always be regarded as synonymous. The resulting diets are referred to as minimum, average or desirable. Since it is at present impossible to define an individual's optimum requirement of any dietary constituent, other methods of evaluating nutritional standards have become necessary. In USA, the recommended dietary allowances² are

designed to provide an adequate diet to cover individual variations among most normal persons of the population. Comparisons between the various standards laid down by national and international bodies are possible only when the purpose of the standards compared is known. In the tables below and on pages 494-495 the standards of the FAO³ and the Joint FAO/WHO Expert Groups⁴⁻⁶ and of the Food and Nutrition Board (USA) are given in detail. For other national standards (British, Canadian, Indian, South African, Australian, Dutch, Norwegian, Central American, Russian) see YOUNG⁷. A new UK standard has recently been published^{8,9}.

Recommended daily allowances of calories (FAO³) and of vitamin A (retinol), thiamine, riboflavin and niacin* (Joint FAO/WHO Expert Group⁴)

Age	Calories per day	Retinol ¹ (mg)	Thiamine (mg)	Riboflavin (mg)	Niacin equivalents ^{1†}
0-3 months**	120 per kg	-	-	-	-
4-6 months**	110 per kg	-	-	-	-
7-12 months	1000	300	0.4	0.6	6.6
1 year	1150	250	0.5	0.6	7.6
2 years	1300	250	0.5	0.7	8.6
3 years	1450	250	0.6	0.8	9.6
4-6 years	1700	300	0.7	0.9	11.2
7-9 years	2100	400	0.8	1.2	13.9
10-12 years	2500	575	1.0	1.4	16.5
13-15 (boys)	3100	725	1.2	1.7	20.4
(girls)	2600	725	1.0	1.4	17.2
16-19 (boys)	3600	750	1.4	2.0	23.8
(girls)	2400	750	1.0	1.3	15.8
Adults (men)	3200	750	1.3	1.8	21.1
(women)	2300	750	0.9	1.3	15.2

* The amounts given do not cover abnormal needs such as those due to infections, malabsorption, metabolic disturbances, extreme environmental conditions, etc. They are also applicable only when the requirements for calories and all other nutrients are fully met.

** For infants from 0 to 6 months, it is accepted that breast feeding by a well-nourished mother is the best way to satisfy the nutritional requirements for these vitamins.

† For diets containing both carotene and retinol, adjustment must be made as follows: recommended intake of mixed vitamin A-active compounds =

$$\text{recommended intake of retinol} \cdot \frac{\beta\text{-carotene } (\mu\text{g})}{\beta\text{-carotene } (\mu\text{g}) + \text{retinol } (\mu\text{g})}, \text{ where } k =$$

1† A niacin equivalent is 1 mg niacin or 60 mg L-tryptophan.

Daily protein requirements (FAO/WHO Expert Group⁴)

Age	Grammes Reference Protein* per kilogramme body weight	
	Average	Range**
Infants†		
0-3 months	2.3	-
3-6 months	1.8	-
6-9 months	1.5	-
9-12 months	1.2	-
Juveniles		
1-3 years	0.88	0.70-1.06
4-6 years	0.81	0.65-0.97
7-9 years	0.77	0.62-0.92
10-12 years	0.72	0.58-0.86
13-15 years	0.70	0.56-0.84
16-19 years	0.64	0.51-0.77
Adults††	0.59	0.47-0.71

* The composition of the Reference Protein is given on page 516. The proteins of eggs, milk and meat all have the same biological value. The intake of proteins of lower biological value must be correspondingly increased.

** This range is based on the expected range of individual variation; the upper level is likely to cover the requirements of 95% of the population and can thus be regarded as a practical allowance.

† In terms of either breast milk or cow's milk protein.

†† During the second and third trimesters of pregnancy 6 g per day should be added, during lactation 15 g per day.

Calories¹

The body requires food energy for resting metabolism, synthesis of body tissues, physical activities, excretory processes and maintenance of thermal balance. The energy requirements at different levels of activity are given in the tables on page 495.

Desirable daily calcium allowances (FAO/WHO Expert Group⁴)

Age	mg/day
0-12 months*	500-600
1-9 years	400-500
10-15 years	600-700
16-19 years	500-600
Adults	400-500
Pregnancy, 3rd trimester	1000-1200
Lactation	1000-1200

* For infants not being breast-fed; the calcium requirement of an infant being breast-fed by a normally lactating mother is met by the breast milk.

The calorie allowances of the Food and Nutrition Board (USA), like those of the FAO, are based on the concept of a 'reference' subject. In the case of the former this is a man aged 22 weighing 70 kg or a woman aged 22 weighing 58 kg with a 'light' level of physical activity in a mean environmental temperature of 20°C. It is assumed that weight gains after this age are likely to be fat. Since it is difficult to estimate the degree of reduction in physical activity associated with advancing age the calorie allowances (see table on page 494) were obtained by making a reduction of 5% between the ages of 22 and 35, of 3% per decade between 35 and 55, and of 5% per decade between 55 and 75. A further reduction of 7% is recommended for persons aged 75 and over. The upper left-hand table on page 495 gives the adjustment for persons of other than the ideal weight.

Increased physical activity also requires an increase in the calorie intake, though more than an additional 1500 kcal over the allowances recommended in the table is rarely necessary. A lower level of physical activity (e.g., in persons with a sedentary occupation) calls for a lower calorie intake.

An environmental temperature lower than 20°C necessitates little if any increase in calorie intake provided that adequate but not

1 1 kcal₁₅ = 4.185 kJ (see page 213).

Nutritional Standards

(For references see page 497)

Recommended daily dietary allowances* (Food and Nutrition Board, USA²)

Age** (years)	Weight		Height		Calories (kcal)	Protein (g)	Minerals																																																																																																																																																																																																																																																																																																																																								
	(kg)	(lb)	cm	(in)			Calcium (g)	Phos- phorus (g)	Iodine (μg)	Iron (mg)	Mag- nesium (mg)																																																																																																																																																																																																																																																																																																																																				
Infants	0-1/2	4 9	55 22		kg × 120	kg × 2.2***	0.4	0.2	25	6	40																																																																																																																																																																																																																																																																																																																																				
	1/2-1	7 15	63 25		kg × 110	kg × 2.0***	0.5	0.4	40	10	60																																																																																																																																																																																																																																																																																																																																				
Children	1-2	9 20	72 28		kg × 100	kg × 1.8***	0.6	0.5	45	15	70																																																																																																																																																																																																																																																																																																																																				
	2-3	12 26	81 32		1100	25	0.7	0.7	55	15	100																																																																																																																																																																																																																																																																																																																																				
	3-4	14 31	91 36		1250	25	0.8	0.8	60	15	150																																																																																																																																																																																																																																																																																																																																				
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	6-8	19 42	110 43		1600	30	0.8	0.8	80	10	200																																																																																																																																																																																																																																																																																																																																				
	8-10	23 51	121 48		2000	35	0.9	0.9	100	10	250																																																																																																																																																																																																																																																																																																																																				
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	14-18	43 95	151 59		2700	50	1.4	1.4	135	18	350																																																																																																																																																																																																																																																																																																																																				
	18-22	59 130	170 67		3000	60	1.4	1.4	150	18	400																																																																																																																																																																																																																																																																																																																																				
	22-35	67 147	175 69		2800	60	0.8	0.8	140	10	400																																																																																																																																																																																																																																																																																																																																				
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Lactation					+ 1000	75	+0.5	+0.5	150	18	450																																																																																																																																																																																																																																																																																																																																				
<table border="1"> <thead> <tr> <th colspan="5">Fat-soluble vitamins</th><th colspan="7">Water-soluble vitamins</th></tr> <tr> <th>Vitamin A activity (IU)</th><th>Vitamin D (IU)</th><th>Vitamin E activity (IU)</th><th>Ascorbic acid (mg)</th><th>Folic acid (mg)</th><th>Niacin†† (mEq)</th><th>Riboflavin (mg)</th><th>Thiamine (mg)</th><th>Vitamin B₆ (mg)</th><th>Vitamin B₁₂ (μg)</th><th colspan="2"></th></tr> </thead> <tbody> <tr> <td>Infants</td><td>0-1/2</td><td>1500</td><td>400</td><td>5</td><td>35</td><td>0.05</td><td>5</td><td>0.4</td><td>0.2</td><td>1.0</td><td></td></tr> <tr> <td></td><td>1/2-1</td><td>1500</td><td>400</td><td>5</td><td>35</td><td>0.05</td><td>7</td><td>0.5</td><td>0.4</td><td>1.5</td><td></td></tr> <tr> <td>Children</td><td>1-2</td><td>1500</td><td>400</td><td>5</td><td>35</td><td>0.1</td><td>8</td><td>0.6</td><td>0.5</td><td>2.0</td><td></td></tr> <tr> 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(μg)			Infants	0-1/2	1500	400	5	35	0.05	5	0.4	0.2	1.0			1/2-1	1500	400	5	35	0.05	7	0.5	0.4	1.5		Children	1-2	1500	400	5	35	0.1	8	0.6	0.5	2.0			2-3	2000	400	10	40	0.1	8	0.6	0.6	2.0			3-4	2000	400	10	40	0.2	8	0.7	0.6	2.5			4-6	2500	400	10	40	0.2	9	0.8	0.7	3			6-8	2500	400	10	40	0.2	11	0.9	0.8	4			8-10	3500	400	15	40	0.2	13	1.1	1.0	4		Males	10-12	4500	400	20	40	0.3	15	1.2	1.1	5			12-14	5000	400	20	40	0.4	17	1.3	1.3	5			14-18	5000	400	20	45	0.4	18	1.4	1.4	5			18-22	5000	400	25	55	0.4	20	1.5	1.5	5			22-35	5000	400	30	60	0.4	18	1.6	1.4	5			35-55	5000	400	30	60	0.4	18	1.7	1.4	5			55-75+	5000	400	30	60	0.4	17	1.7	1.3	5		Females	10-12	4500	400	20	40	0.4	14	1.7	1.2	6			12-14	5000	400	20	45	0.4	15	1.3	1.1	5			14-16	5000	400	20	45	0.4	15	1.4	1.2	5			16-18	5000	400	25	50	0.4	16	1.4	1.2	5			18-22	5000	400	25	55	0.4	15	1.5	1.2	5			22-35	5000	400	25	55	0.4	13	1.5	1.0	5			35-55	5000	400	25	55	0.4	13	1.5	1.0	5			55-75+	5000	400	25	55	0.4	13	1.5	1.0	5		Pregnancy		6000	400	30	60	0.8	15	1.8	+0.1	8		Lactation		8000	400	30	60	0.5	20	2.0	+0.5	6	
Fat-soluble vitamins					Water-soluble vitamins																																																																																																																																																																																																																																																																																																																																										
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Children	1-2	1500	400	5	35	0.1	8	0.6	0.5	2.0																																																																																																																																																																																																																																																																																																																																					
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	4-6	2500	400	10	40	0.2	9	0.8	0.7	3																																																																																																																																																																																																																																																																																																																																					
	6-8	2500	400	10	40	0.2	11	0.9	0.8	4																																																																																																																																																																																																																																																																																																																																					
	8-10	3500	400	15	40	0.2	13	1.1	1.0	4																																																																																																																																																																																																																																																																																																																																					
Males	10-12	4500	400	20	40	0.3	15	1.2	1.1	5																																																																																																																																																																																																																																																																																																																																					
	12-14	5000	400	20	40	0.4	17	1.3	1.3	5																																																																																																																																																																																																																																																																																																																																					
	14-18	5000	400	20	45	0.4	18	1.4	1.4	5																																																																																																																																																																																																																																																																																																																																					
	18-22	5000	400	25	55	0.4	20	1.5	1.5	5																																																																																																																																																																																																																																																																																																																																					
	22-35	5000	400	30	60	0.4	18	1.6	1.4	5																																																																																																																																																																																																																																																																																																																																					
	35-55	5000	400	30	60	0.4	18	1.7	1.4	5																																																																																																																																																																																																																																																																																																																																					
	55-75+	5000	400	30	60	0.4	17	1.7	1.3	5																																																																																																																																																																																																																																																																																																																																					
Females	10-12	4500	400	20	40	0.4	14	1.7	1.2	6																																																																																																																																																																																																																																																																																																																																					
	12-14	5000	400	20	45	0.4	15	1.3	1.1	5																																																																																																																																																																																																																																																																																																																																					
	14-16	5000	400	20	45	0.4	15	1.4	1.2	5																																																																																																																																																																																																																																																																																																																																					
	16-18	5000	400	25	50	0.4	16	1.4	1.2	5																																																																																																																																																																																																																																																																																																																																					
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	55-75+	5000	400	25	55	0.4	13	1.5	1.0	5																																																																																																																																																																																																																																																																																																																																					
Pregnancy		6000	400	30	60	0.8	15	1.8	+0.1	8																																																																																																																																																																																																																																																																																																																																					
Lactation		8000	400	30	60	0.5	20	2.0	+0.5	6																																																																																																																																																																																																																																																																																																																																					

* The allowance levels are intended to cover individual variations among most normal persons as they live in the USA under usual environmental stresses. The recommended allowances can be attained with a variety of common foods providing other nutrients for which human requirements have been less well defined.

** Entries on lines for age range 22-35 years represent the 'reference' man and woman at age 22 (see under 'Calories', page 493). All other entries represent allowances for the midpoint of the specified age range.

*** Assumes protein equivalent to human milk. For proteins not 100% utilized, factors should be increased proportionately.

† The folic acid allowances refer to dietary sources as determined by *Lactobacillus casei* assay. Pure forms of folic acid may be effective in doses less than a quarter of the recommended allowance.

†† Niacin equivalents include dietary sources of the vitamin itself plus 1 mEq for each 60 mg of dietary tryptophan.

Recommended daily calorie allowances (Food and Nutrition Board, USA²) (Light physical activity, mean energy expenditure)

	Body weight		RMR* at age 22
	(kg)	(lb)	
Men	50	110	1540
	55	121	1620
	60	132	1720
	65	143	1820
	70	154	1880
	75	165	1970
	80	176	2020
	85	187	2110
	90	198	2210
	95	209	2290
	100	220	2380
Women	40	88	1280
	45	99	1380
	50	110	1460
	55	121	1560
	58	128	1620
	60	132	1640
	65	143	1740
	70	154	1830

* RMR = resting metabolic rate, approximate rate measured under basal conditions.

Energy requirements during various activities

Light work (2.5-4.9 kcal/min)	Moderately heavy work (5.0-7.4 kcal/min)
Light industrial and domestic work	Farm work
Gymnastics	Marching with pack
Tile-laying	Ballroom dancing
Painting	Playing tennis
Tending agricultural machines	Cycling
Driving goods vehicles	
Playing golf, bowling	

unreasonably heavy clothing is worn creases with increasing environmental temperatures between 30°C and 40°C the caloric requirement is 0.5% per 1°C, but only when physical activity is present.

The Food and Nutrition Board (infants and children are average and 2 in the case of adult allowances, they accordance with observations of appropriate growth as judged by amount of subcutaneous fat.

The calorie allowances recommended here are higher than those of the Food and Nutrition Board for an assumed greater physical activity in various activities can be calculated on this page.

Protein

Dietary protein is the source of the amino acids, i.e., those necessary for the synthesis of other nitrogenous substances (see the protein allowance for adults recommended by the Food and Nutrition Board (USA)² (page 494), based on food protein, is 0.9 g protein per 100 kcal of food energy. In the case of infants it is as normal sufficient protein is obtained through this provides little more than for the protein allowances recommended by the Food and Nutrition Board (see page 493). For premature

Recommended daily calorie allowances (Food and Nutrition Board, USA²) (Light physical activity, mean environmental temperature 20°C)

Body weight (kg) (lb)	RMR* at age 22	Age		
		22	45	65
Men				
50	110	1540	2200	2000
55	121	1620	2350	2150
60	132	1720	2500	2300
65	143	1820	2650	2400
70	154	1880	2800	2500
75	165	1970	2950	2600
80	176	2020	3050	2700
85	187	2110	3200	2800
90	198	2210	3350	2900
95	209	2290	3500	3000
100	220	2380	3700	3100
Women				
40	88	1280	1550	1450
45	99	1380	1700	1550
50	110	1460	1800	1650
55	121	1560	1950	1750
58	128	1620	2000	1850
60	132	1640	2050	1900
65	143	1740	2200	2000
70	154	1830	2300	2100

* RMR = resting metabolic rate, approximately 10% above the metabolic rate measured under basal conditions.

Energy requirements during various activities⁷

Light work (2.5-4.9 kcal/min)	Moderately heavy work (5.0-7.4 kcal/min)	Heavy work (7.5-9.9 kcal/min)	Very heavy work (over 10 kcal/min)
Light industrial and domestic work	Farm work	Mine- working	Felling trees
Gymnastics	Marching with pack	Playing football	Steelmaking
Tile-laying	Ballroom dancing		Swimming (crawl)
Painting	Playing tennis		Climbing
Tending agricul- tural machines	Cycling		
Driving goods vehicles			
Playing golf, bowling			

unreasonably heavy clothing is worn. The calorie requirement increases with increasing environmental temperature; at temperatures between 30°C and 40°C the calorie intake should be raised by 0.5% per 1°C, but only when physical activity is undiminished.

The Food and Nutrition Board (USA) calorie allowances for infants and children are average and approximate. Even more than in the case of adult allowances, they may need to be adjusted in accordance with observations of appetite, activity and nature of growth as judged by amount of subcutaneous fat.

The calorie allowances recommended by the FAO (page 493) are higher than those of the Food and Nutrition Board (USA) as a result of an assumed greater physical activity. Energy requirements during various activities can be calculated by means of the tables on this page.

Protein

Dietary protein is the source of the nitrogen and essential amino acids, i.e., those necessary for the synthesis of the body proteins and other nitrogenous substances (see the table opposite). The dietary protein allowance for adults recommended by the Food and Nutrition Board (USA)² (page 494), based on a utilization value of 70% for food protein, is 0.9 g protein per kilogramme body weight per day, a value about nine times the minimum requirement of Reference Protein⁴. In the case of infants it is assumed that when lactation is normal sufficient protein is obtained from the breast milk, even though this provides little more than the minimum requirement. For the protein allowances recommended by the FAO/WHO Expert Group see page 493. For premature infants the recommended

Normal energy requirements of recumbent adults⁷

Body weight			kg lb	45 99	50 110	55 121	60 132	65 143	70 154	75 165	80 176
Fat (%)	Body type		Energy requirement (kcal/min)								
	Men	Women									
5-9	Lean	-	-	0.99	1.06	1.12	1.19	1.26	1.32	1.39	
10-14	Average	-	-	0.94	1.01	1.08	1.14	1.21	1.28	1.34	
15-19	Heavily built	Lean	0.82	0.89	0.96	1.03	1.09	1.16	1.23	1.30	
20-24	Corpulent	Average	0.78	0.84	0.91	0.98	1.05	1.11	1.18	1.25	
25-29	-	Heavily built	-	0.80	0.86	0.93	1.00	1.07	1.13	1.20	
>30	-	Corpulent	-	-	0.81	0.88	0.95	1.02	1.08	1.15	

Normal energy requirements of adults sitting and at rest⁷

Age in years	Number of subjects	Energy requirement (kcal/min)		
		Mean	Range	t
Men (65 kg = 143 lb)				
20-39	30	1.39	0.97-1.79	0.25
40-64	30	1.37	0.87-1.94	0.29
65 and over	23	1.29	0.91-1.94	0.25
Women (55 kg = 121 lb)				
20-39	30	1.15	0.75-1.68	0.28
40-59	30	1.07	0.78-1.56	0.19
60 and over	23	1.09	0.77-1.62	0.31

Normal energy requirements of adults during walking⁷

Body weight	kg	45	55	65	75	85	95
	lb	99	121	143	165	187	209
	Energy requirement (kcal/min)						
Rate of walking							
km/h	mi/h						
3	1.9	2.1	2.5	2.8	3.2	3.5	3.8
4	2.5	2.7	3.2	3.6	4.0	4.3	4.6
5	3.1	3.2	3.7	4.2	4.7	5.1	5.5
6	3.7	3.8	4.4	4.9	5.4	5.9	6.4
7	4.3	4.4	5.0	5.5	6.1	6.6	7.1

allowance* is between 2.5 g and a maximum of 6 g per kilogramme body weight per day.

Carbohydrate and fat²

The desirable intake of carbohydrate and fat, like the optimal fatty-acid composition of foods, is difficult to assess. Apart from the body's specific needs for carbohydrate (e.g., brain energy), this and fat appear to be interchangeable as dietary energy sources, and in the body they are interconvertible, except that fatty acids with an even number of C-atoms from neutral fat, etc. cannot be used to form carbohydrate. Adaptation to diets very low in carbohydrate is possible, but for persons accustomed to a normal diet at least 100 g carbohydrate per day appears to be necessary if metabolic disturbances like ketosis, excessive protein breakdown, etc. are to be avoided. Normally, less than 1800 kcal of carbohydrate is stored as glycogen, so that if there is not enough in the diet it must be derived largely from dietary or body protein.

Dietary fat is also a carrier of other nutrients, including vitamins A, D, E and K. Calories, whether derived from dietary carbohydrate or fat, are stored mainly as fat in adipose cells. Except for the central nervous system, almost all body tissues utilize fatty acids directly as a source of energy.

The polyunsaturated fatty acids like linoleic and arachidonic acids have been shown to be essential for animals, and are very probably also essential for man. The minimum human requirements of the essential fatty acids should probably represent about 2% of the total calorie intake⁹, or about 2.4 g linoleic acid per 1000 kcal of nutrient intake. In infants, a subclinical deficiency of linoleic acid

Minerals			
Phosphorus (g)	Iodine (μg)	Iron (mg)	Magnesium (mg)
0.2	25	6	40
0.4	40	10	60
0.5	45	15	70
0.7	55	15	100
0.8	60	15	150
0.8	70	10	200
0.8	80	10	200
0.9	100	10	250
1.0	110	10	250
1.2	125	10	300
1.4	135	18	350
1.4	150	18	400
0.8	140	10	400
0.8	140	10	350
0.8	125	10	350
0.8	110	10	350
1.2	110	18	300
1.3	115	18	350
1.3	120	18	350
1.3	115	18	350
0.8	100	18	350
0.8	100	18	300
0.8	90	18	300
0.8	80	10	300
1.4	125	18	450
1.5	150	18	450

Vitamin B₁₂

Thiamine (mg)	Vitamin B ₁₂ (μg)	Vitamin B ₆ (μg)
0.2	0.2	1.0
0.4	0.3	1.5
0.5	0.4	2.0
0.6	0.5	2.0
0.6	0.6	2.5
0.7	0.7	3
0.8	0.9	4
1.0	1.0	4
1.1	1.2	5
1.3	1.4	5
1.4	1.6	5
1.5	1.8	5
1.4	2.0	5
1.4	2.0	5
1.3	2.0	5
1.2	2.0	6
1.1	1.4	5
1.2	1.6	5
1.2	1.8	5
1.2	2.0	5
1.0	2.0	5
1.0	2.0	5
1.0	2.0	5
1.0	2.0	6
+0.1	2.5	8
+0.5	2.5	6

human milk. For proteins not 100% proportionately.

Lactation sources as determined by Lactation may be effective in doses less than

sources of the vitamin itself plus 1 mEq

can be prevented by ensuring that the amount of this substance in the formula supplies 3% of the calories¹⁰.

Essential amino acids

Of the 18 amino acids contained in food proteins 8 are essential in that the body is not capable of synthesizing them (tryptophan, phenylalanine, lysine, threonine, methionine, leucine, isoleucine, valine), 2 are semi-essential in that they are not synthesized in adequate amounts during growth (histidine, arginine), and 6 are non-essential in that the body can synthesize them from a nitrogen source such as any amino acid, ammonium salts or urea (aspartic acid, glutamic acid, proline, glycine, serine, alanine).

Requirements of the essential amino acids*

Amino acid	Infants Minimum require- ment ^{††} (mg/kg/ day)	Adults**		Recom- mended intake ^{†‡} (g/day)
		Minimum requirement		
		Young men ^{‡‡} (g/day)	Young women ^{‡‡} (g/day)	
L-Histidine	34	0	0	0
L-Tryptophan	22	0.25	0.16	0.50
L-Phenylalanine				
Tyrosine available [†]	90	0.30	0.22	—
Tyrosine not available	—	1.10	—	2.20
L-Lysine	103	0.80	0.50	1.60
L-Threonine	87	0.50	0.31	1.00
L-Methionine				
Cystine available ^{††}	45	0.20	0.35	—
Cystine not available	—	1.10	—	2.20
L-Leucine	150	1.10	0.62	2.20
L-Isoleucine	126	0.70	0.45	1.40
L-Valine	105	0.80	0.65	1.60

* Assuming that the nitrogen intake is adequate for the formation of the non-essential amino acids.

** The requirements are higher during pregnancy and lactation. The minimum requirement of men over 50 is higher than that of young men in respect of at least two amino acids (methionine 2.4–3.0 g/day, lysine 1.4–2.8 g/day)^{††}.

† 70–75% of the phenylalanine requirement can be met by tyrosine^{‡‡}.

†† 80–90% of the methionine requirement can be met by cystine^{‡‡}.

Water

The water requirement of the body is determined by the amount of heat it produces and by the load of solutes in the body fluids. It is closely linked to the intake of salt. The intake must replace water losses in the urine, faeces, sweat and insensible perspiration (skin and lungs). Under the most favourable conditions (low-solute diet, resting, no sweating) the total water supplied by the diet and metabolic processes should be at least 1.5 l/day¹⁶. A reasonable water allowance is 1 ml per calorie of food². In hot, dry climates the water requirement can be considerably increased as a result of sweating. Under ordinary conditions, infants require proportionately more water than adults and should be given 1.5 ml per calorie of food².

See also 'Water and Electrolyte Balance', pages 523–530.

Sodium and chloride

The requirement of sodium and chloride is closely linked to the water balance of the body. Both the total body content and body-fluid concentration of sodium are homeostatically controlled, moderate intakes being rapidly excreted in the urine while a reduction in intake causes excretion to drop quickly to a very low level¹⁶. Sodium deficiency is rare in healthy persons provided there is no abnormal loss. The normal recommended NaCl intake is 1 g per kilogramme of water². Hard physical work in the tropics would require a daily intake of up to 19 g NaCl¹⁷. Normal diets in western Europe and USA contain 6–18 g NaCl.

Potassium

The minimum daily potassium requirement probably amounts to 0.8–1.3 g². Normal diets in western countries provide 0.8–1.5 g potassium per 1000 kcal. An adequate potassium intake is important during prolonged intravenous feeding, recovery from severe

diarrhoea, and diabetic acidosis; this should be met by an intake of 40–120 mg (1–3 mEq) per kg body weight per day.

Magnesium

The probable daily magnesium requirement¹⁸ is 150 mg for children under 10 years and 200–300 mg for older children; with a daily protein intake of 70–80 g, men require 300–400, women 300 mg per day. The requirement seems to increase with increasing protein intake. For the recommendations of the Food and Nutrition Board (USA) see the table on page 494. An average diet provides 250–500 mg magnesium per day.

Calcium and phosphorus

Knowledge of the minimum requirements of calcium is inadequate, but it is well established that no injurious effects occur when the daily calcium intake lies between 300 mg and 2000 mg¹⁹.

The desirable calcium intakes (*suggested practical allowances*) recommended by the FAO/WHO Expert Group (see table on page 493) are lower than those of the Food and Nutrition Board (USA)² (see table on page 494).

Although the calcium-phosphorus ratio in bone is 2:1 it is much lower in the soft tissues. This, and the fact that on a normal diet the intake of phosphorus always equals or exceeds that of calcium, have led to the recommendation² that the calcium and phosphorus allowances should be equal except in the case of young infants (see the table on page 494).

Iron

In order to remain in iron balance, the daily iron intake of men, as well as of women after the menopause, must be 0.5–1.0 mg; menstruating women require some 0.3–1.0 mg per day more²⁰. The iron requirement is increased during pregnancy, particularly as a result of the increase in the total erythrocyte volume, and this need is met partly by mobilization of iron reserves. The latter are restored post partum when the total erythrocyte volume falls again.

During the first months of life the iron requirement of infants is met principally from endogenous sources. Special attention should be paid to the iron needs of growing girls, who have to meet not only the requirements of growth but also cover menstruation losses. Assuming that 10% of the dietary iron is absorbed, the following amounts must be available in the diet:

Dietary iron requirements (Committee on Iron Deficiency Anemia, American Medical Association²¹)

	Iron require- ment of body mg/day	Dietary iron content* mg/day
Men	0.5–1.0	5–10
Menstruating women	0.7–2.0	7–20
Pregnant women	2.0–4.8	20–48
Adolescents	1.0–2.0	10–20
Children	0.4–1.0	4–10
Infants	0.5–1.5	1.5 mg/kg**

* Assuming 10% absorption of dietary iron.

** Up to a maximum of 15 mg.

With the exception of those during pregnancy, these requirements are almost identical with the recommendation of the Food and Nutrition Board (USA) (page 494).

Estimates of dietary iron intake in the UK, USA, Australia and Canada have given values of 10–20 mg per person per day; various studies in the USA have shown that children aged 3–6 years have a daily iron intake of 3–11 mg²².

In infants the iron requirement is not met by the normal intake of breast or cow's milk alone. Premature infants, as well as infants with iron deficiency, require additional iron from the 2nd to 3rd month of life on; this should be given in the form of enriched cereal products or possibly iron salts²³. The iron requirement in pregnancy is likewise met only with difficulty from dietary sources.

Copper

Copper is an important component of various enzymes involved in oxygen transport. In USA and Europe the diet of adults contains an average of 1–5 mg copper per day²⁴. The daily requirement of adults has been estimated at 1.5–2 mg^{2, 25}, that of infants and children at 0.04–0.14 mg per kilogramme body weight²⁴.

Manganese

Manganese is very probably an essential element. It appears to be involved in the synthesis of phospholipids²⁶. The daily diet of an adult apparently sufficient to maintain a positive manganese balance is 0.2–0.3 mg manganese per kilogramme body weight²⁷.

Zinc

Zinc is an important component of many enzymes. The daily dietary intake of zinc is 10–15 mg²⁸. Zinc milk falls during the course of lactation inadequate to meet the infant's zinc requirements.

Cobalt

Cobalt is a component of vitamin B₁₂ not used by the human body in any form other than as cobalt salts of doubtful value.

Molybdenum

Molybdenum is an essential component of several enzymes, particularly xanthine oxidase²⁹. The minimum sufficient molybdenum to meet the needs of adults is 0.05 mg³⁰.

Vanadium

Vanadium is possibly an essential element involved in lipid metabolism. The daily requirement is 0.5–1.0 mg³¹.

Chromium

Chromium in its trivalent form is present in the human body in small amounts. It is essential for insulin function³². The daily dietary intake of which about 1% is absorbed, enough to meet the needs of adults is 0.1 mg³³.

Selenium

Selenium has not been shown to be essential for human health, although a role in the metabolism of toxic substances has been suggested³⁴.

Iodine

Iodine is a component of thyroid hormones. The minimum requirement of adults is 0.1 mg per day; children and pregnant women require 0.2–0.5 mg³⁵. On the basis of the iodine turnover studies the optimum requirement of adults has been estimated at 0.1 mg³⁶. For the recommendations of the FAO/WHO Expert Group (see table on page 494) see the table on page 494. The iodine content of the soil from which the food is produced is of great importance for the iodine intake of the population (as potassium iodide) is probably sufficient to meet the needs of the population in most countries. On the average, iodine intake is 0.1 mg per day in the USA, 0.2 mg in the UK, 0.3 mg in the Republic of Poland, 0.4 mg in Switzerland, 0.5 mg in the USSR, 0.6 mg in the Netherlands, 0.7 mg in the Federal Republic of Germany, 0.8 mg in the Republic of France, 0.9 mg in the Republic of Austria, 1.0 mg in the Republic of Italy, 1.1 mg in the Republic of Spain, 1.2 mg in the Republic of Greece, 1.3 mg in the Republic of Portugal, 1.4 mg in the Republic of Belgium, 1.5 mg in the Republic of Luxembourg, 1.6 mg in the Republic of Denmark, 1.7 mg in the Republic of Sweden, 1.8 mg in the Republic of Norway, 1.9 mg in the Republic of Finland, 2.0 mg in the Republic of Iceland, 2.1 mg in the Republic of Ireland, 2.2 mg in the Republic of Malta, 2.3 mg in the Republic of Cyprus, 2.4 mg in the Republic of Turkey, 2.5 mg in the Republic of Greece, 2.6 mg in the Republic of Bulgaria, 2.7 mg in the Republic of Romania, 2.8 mg in the Republic of Hungary, 2.9 mg in the Republic of Czech Republic, 3.0 mg in the Republic of Slovakia, 3.1 mg in the Republic of Poland, 3.2 mg in the Republic of Czech Republic, 3.3 mg in the Republic of Slovakia, 3.4 mg in the Republic of Hungary, 3.5 mg in the Republic of Poland, 3.6 mg in the Republic of Czech Republic, 3.7 mg in the Republic of Slovakia, 3.8 mg in the Republic of Hungary, 3.9 mg in the Republic of Poland, 4.0 mg in the Republic of Czech Republic, 4.1 mg in the Republic of Slovakia, 4.2 mg in the Republic of Hungary, 4.3 mg in the Republic of Poland, 4.4 mg in the Republic of Czech Republic, 4.5 mg in the Republic of Slovakia, 4.6 mg in the Republic of Hungary, 4.7 mg in the Republic of Poland, 4.8 mg in the Republic of Czech Republic, 4.9 mg in the Republic of Slovakia, 5.0 mg in the Republic of Hungary, 5.1 mg in the Republic of Poland, 5.2 mg in the Republic of Czech Republic, 5.3 mg in the Republic of Slovakia, 5.4 mg in the Republic of Hungary, 5.5 mg in the Republic of Poland, 5.6 mg in the Republic of Czech Republic, 5.7 mg in the Republic of Slovakia, 5.8 mg in the Republic of Hungary, 5.9 mg in the Republic of Poland, 6.0 mg in the Republic of Czech Republic, 6.1 mg in the Republic of Slovakia, 6.2 mg in the Republic of Hungary, 6.3 mg in the Republic of Poland, 6.4 mg in the Republic of Czech Republic, 6.5 mg in the Republic of Slovakia, 6.6 mg in the Republic of Hungary, 6.7 mg in the Republic of Poland, 6.8 mg in the Republic of Czech Republic, 6.9 mg in the Republic of Slovakia, 7.0 mg in the Republic of Hungary, 7.1 mg in the Republic of Poland, 7.2 mg in the Republic of Czech Republic, 7.3 mg in the Republic of Slovakia, 7.4 mg in the Republic of Hungary, 7.5 mg in the Republic of Poland, 7.6 mg in the Republic of Czech Republic, 7.7 mg in the Republic of Slovakia, 7.8 mg in the Republic of Hungary, 7.9 mg in the Republic of Poland, 8.0 mg in the Republic of Czech Republic, 8.1 mg in the Republic of Slovakia, 8.2 mg in the Republic of Hungary, 8.3 mg in the Republic of Poland, 8.4 mg in the Republic of Czech Republic, 8.5 mg in the Republic of Slovakia, 8.6 mg in the Republic of Hungary, 8.7 mg in the Republic of Poland, 8.8 mg in the Republic of Czech Republic, 8.9 mg in the Republic of Slovakia, 9.0 mg in the Republic of Hungary, 9.1 mg in the Republic of Poland, 9.2 mg in the Republic of Czech Republic, 9.3 mg in the Republic of Slovakia, 9.4 mg in the Republic of Hungary, 9.5 mg in the Republic of Poland, 9.6 mg in the Republic of Czech Republic, 9.7 mg in the Republic of Slovakia, 9.8 mg in the Republic of Hungary, 9.9 mg in the Republic of Poland, 10.0 mg in the Republic of Czech Republic.

Fluorine

Fluorine is a component of dental enamel and provides protection against dental caries³⁷. The minimum daily fluoride intake for adults is 1 mg fluoride per litre of the incident water³⁸. In children aged 6–15 years, is less than 1 mg/l the daily fluoride intake has been estimated at 0.4–0.8 mg for children aged 6–15 years. The Council on Nutrition has recommended 0.25 mg for infants and 0.5–1.0 mg for children. In areas where the fluoride content of

